JOURNAL
OF THE
FISHERIES RESEARCH BOARD OF CANADA

Volume 16

1959

Editor:

W. E. RICKER
Fisheries Research Board of Canada
P.O. Drawer 100
Nanaimo, B.C., Canada

Associate Editor:

N. M. CARTER
Fisheries Research Board of Canada
Room 32, West Block
Ottawa, Ont., Canada

Manuscripts for publication should be addressed to the Editor

QUEEN'S PRINTER AND CONTROLLER OF STATIONERY
OTTAWA, 1959

CONTENTS

No. 1. (*Published January 15, 1959*)

No. 2. (*Published March 15, 1959*)

NOTE

No. 3. (*Published May 30, 1959*)

NOTES

No. 4. (*Published August 31, 1959*)

NOTES

No. 5. (*Published November 20, 1959*)

NOTES

No. 6. (*Published December 31, 1959*)

JOURNAL
OF THE
FISHERIES RESEARCH BOARD OF CANADA

Volume 16, No. 1

CONTENTS

Continued Overleaf

OTTAWA
JANUARY
1959

W. E. RICKER
N. M. CARTER
Editors

QUEEN'S PRINTER AND CONTROLLER OF STATIONERY
OTTAWA, 1959

Effect of Chlortetracycline (Aureomycin) on the Keeping Quality of Freshwater Fish under Tropical Conditions[1]

By K. Visweswariah, M. N. Moorjani, D. S. Bhatia and V. Subrahmanyan

Central Food Technological Research Institute, Mysore, India

ABSTRACT

Chlortetracycline had no effect in prolonging the keeping quality of round and eviscerated freshwater fish when stored at 30°C. However, the effectiveness of the antibiotic in prolonging the storage life of fish fillets stored under identical conditions was demonstrated.

INTRODUCTION

Bacterial spoilage normally accounts for much greater economic losses than any of the other well-known causes of fish spoilage such as oxidative and enzymic action. The composition of the bacterial flora varies due to differing environment, species, fishing season, and other factors. It has been established that the antibiotic chlortetracycline (Aureomycin; CTC) is the most effective of different bacteriostatic agents in controlling microbial spoilage in fish (Tarr *et al.*, 1950, 1952, 1954; Farber, 1954; Tarr, 1956, 1957; Southcott *et al.*, 1958). The special advantages in the application of this antibiotic are that it is non-toxic, economic, and is rapidly destroyed by normal cooking of fish flesh that has been treated with it (Boyd *et al.*, 1957). Though CTC has a broad antibacterial spectrum, its effect will be influenced by the composition of bacterial flora and the storage temperatures.

Since extension of storage life of fresh fish may be very helpful to fishermen who lack any cold storage facilities, we undertook to investigate the effect of CTC in prolonging the keeping quality of round and eviscerated fish, and fish fillets, during storage at a comparatively high temperature (30°C) such as is normally experienced under tropical conditions.

EXPERIMENTAL

The freshwater fish of species mentioned later were caught by net from tanks and the neighbouring Cauvery River where water temperatures averaged 20°C. They were iced shortly after catching and transported to the laboratory in iced condition within 2 hours. The fish were eviscerated or filleted in the laboratory under hygienic conditions.

Antibiotic. The CTC used was in the form of Aureomycin hydrochloride 16.5% (Acronize B_1)[2].

Fish treatment. Within 1 to 2 hours of being received in the laboratory, some of each lot of fish were eviscerated, some were processed into skinless fillets, and the remainder were left in the round state. Part of the samples so prepared were

[1]Received for publication April 8, 1958.

[2]Kindly supplied free of cost by M/s. Lederle Laboratories, Bombay, India.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

used as untreated controls for the antibiotic-treated samples. For the round and eviscerated fish the treatment consisted of a dip of from 1 to 6 hours in CTC solutions of concentrations ranging from 25 to 100 ppm. The skinless fillets were dipped in a 5-ppm CTC solution for 1 hour. The treated and untreated samples were sealed in alkathene bags and incubated at 30°C for periodic examination.

Evaluation of the condition of fish. The treated and untreated samples were judged organoleptically at intervals till they became unfit for human consumption. In the case of the eviscerated fish, the head, scales and fins were removed, and from the round fish the intestines were also removed. The remaining muscle was minced and pressed in a Carver press to obtain press juice, which was then centrifuged and assayed for total volatile base (TVB) (Conway, 1947) and total volatile reducing substances (VRS) (Farber, 1949). For viable bacterial counts, a 5-g portion of fillet was cut under aseptic conditions and blended with 45 ml of sterile water in a tissue homogenizer. Suitable dilutions were plated using nutrient agar (beef extract 0.3%, peptone 0.5%, agar 1.5%, at pH 7.0). The plates were incubated at 25°C for a period of 3 days, colonies being counted on those which had between 30 and 300.

RESULTS

Both the treated and untreated samples of scaly fish, *Barbus carnaticus* and *Barbus dubius* (weighing 200 to 240 g), that had been given a 1-hour dip in 5-ppm CTC kept well when left in the round state for 6 hours, as revealed by organoleptic and TVB data; a slight sour odour developed at the end of this period, indicating the onset of spoilage (Fig. 1). The sour odour became more intense with time and both treated and untreated samples were inedible after about 12 hours. Even prolonged dip treatment of round fish in 25 to 100 ppm CTC for 1 to 6 hours did not reveal any beneficial effect on the keeping quality, probably because the antibiotic could not pentrate into the muscle through the thick skin and well developed scales.

The work was repeated on "Godle" (*Callichrous bima-culatus*), a non-scaly fish. Even in this study, the treated samples did not exhibit any appreciable improvement in their keeping quality.

Eviscerated *Barbus carnaticus* and a few other species (weighing 250 to 400 g) were given a 1-hour dip in CTC solutions of concentration ranging from 25 to 100 ppm. Both treated fish and controls were held at 30°C. Though the eviscerated fish, both treated and untreated, kept well for 8 to 10 hours, they were not in an edible condition after 16 to 18 hours at that temperature. The treated samples did not reveal any beneficial effect (Fig. 1) possibly because the antibiotic could not penetrate the hard thick membrance lining the visceral cavity.

Experiments were extended to include fillets of "Korva" and "Avalu" (double breathers) belonging to species of *Ophicephalus*. It was observed that the initial TVB values of flesh from freshly caught "Korva" and "Avalu" were 16.0 and 12.0 mg N/%, respectively. These high initial TVB values were almost in the range (17 to 14) found for spoiled samples of other freshwater fish (Moorjani *et al.*, 1958).

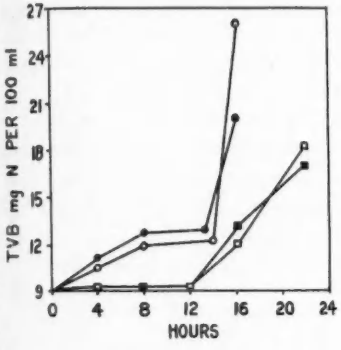

FIG. 1. Effect of Aureomycin (CTC) on the keeping quality of round and eviscerated "Gende" (*Barbus carnaticus*) at 30°C.
- ○ Round, control
- □ Eviscerated, control
- ● Round, treated
- ■ Eviscerated, treated

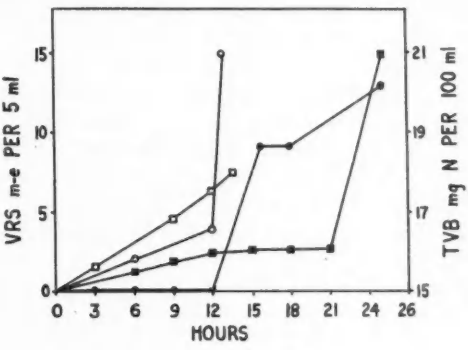

FIG. 2. Effect of Aureomycin (CTC) on the keeping quality of "Korva" (*Ophicephalus*) fillets at 30°C.
- ○ VRS, control
- □ TVB, control
- ● VRS, treated
- ■ TVB, treated

Skinless "Korva" fillets were given a 1-hour dip in CTC solutions of concentration ranging from 5 to 50 ppm. It was observed from organoleptic, TVB and VRS data that the keeping quality was not much influenced with progressive increase in the concentration of the antibiotic and that a 1-hour dip in as low a concentration as 5 ppm was quite adequate. The organoleptic tests correlated well with TVB, VRS and bacterial number. The control fish fillets developed a slight

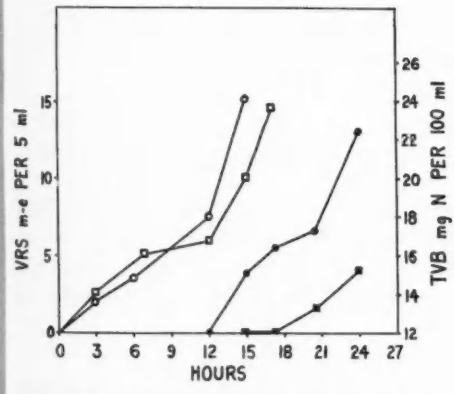

FIG. 3. Effect of Aureomycin (CTC) on the keeping quality of "Avalu" (*Ophicephalus*) fillets at 30°C.
- ○ VRS, control
- □ TVB, control
- ● VRS, treated
- ■ TVB, treated

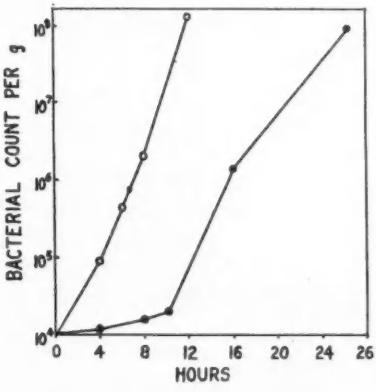

FIG. 4. Effect of Aureomycin (CTC) on bacterial growth of "Korva" fillets at 30°C.
- ○ Control
- ● Treated

sour odour at the ninth hour and became inedible at the twelfth hour of holding at 30°C. By the latter time there were over 10^8 bacteria per gram of flesh (VRS value of 15.0 m-e and TVB value 20.0 mg N/%). The treated fillets attained this state only after 22 to 24 hours, thereby indicating an increase of 10 to 12 hours of storage life (Fig. 2). Figure 3 relates to similar studies on "Avalu" fish. The control samples started developing a slight sour odour about the eighth hour and became totally unacceptable after 12 hours. The treated fillets reached this state of spoilage only after 22 to 24 hours. The pronounced effect of CTC in suppressing bacterial growth is also revealed in Fig. 4 and 5.

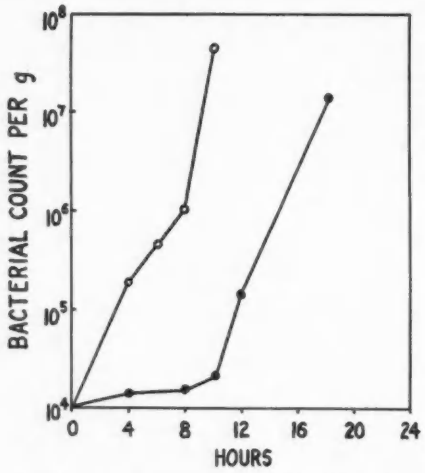

FIG. 5. Effect of Aureomycin (CTC) on bacterial growth of "Avalu" fillets at 30°C.
○ Control
● Treated

Thus, the effectiveness of CTC in prolonging the storage life of fish fillets is demonstrated for "Korva" and "Avalu" varieties. The effect will probably be more pronounced at lower temperatures.

The uptake of CTC by fillets was estimated by a spectrophotometric method (Chiccarelli, *et al.*, 1957). It was found that 1 g of raw fish exhibited an uptake of 1.3 μg of CTC when dip treatment was for 1 hour in 5-ppm solution. This level of uptake is too small to be of any consequence from the public health point of view.

SUMMARY

Round and eviscerated freshwater fish when given a dip treatment of from 1 to 6 hours in 25 to 100 ppm of CTC and stored at 30°C did not show any improvement in their keeping quality as revealed by organoleptic and chemical evaluation. On the other hand a dip treatment in a 5-ppm CTC solution for 1 hour almost doubled the storage life of fillets from such fish.

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1952. *Food Technol.*, **6**: 363–366.

The Chemical Composition of Sea Water in the Vicinity of the Atlantic Provinces of Canada[1]

BY E. GORDON YOUNG AND D. G. SMITH

Atlantic Regional Laboratory,
National Research Council, Halifax, N.S.

AND

W. M. LANGILLE

Department of Chemistry,
Nova Scotia Agricultural College, Truro, N.S.

ABSTRACT

Samples of sea water from eight locations at the surface around the coast of the Atlantic Provinces of Canada have been analyzed for their content of major and minor chemical constituents. The salt water in the Bras d'Or Lakes of Cape Breton Island, N.S., was different from that in the contiguous Atlantic Ocean and showed evidence of much dilution. The seven other samples examined averaged 17.17‰ for chlorinity and 31.03‰ for salinity. These averages are low for open oceanic waters. The average composition of sea water for this area was as follows in grams per kilogram: Na, 9.55; K, 0.34; Ca, 0.37; Mg, 1.15; SO_4, 2.36; B as H_3BO_3, 0.0243. Concentrations of the trace elements in micrograms per litre varied within the following limits: As as As_2O_5, 1.4 to 2.0; Co, 0.33 to 0.67; Cu, 13 to 22; F, 860 to 1200; I, 6 to 53; Mo, 6.3 to 14.0; PO_4, 5 to 69; Si, 44 to 95; Zn, 6.5 to 10.9. Nickel was also present in all samples but vanadium was not detected. The various ratios of the mineral elements, especially to chlorine, have been calculated, and show only slight divergence from those for open ocean water.

INTRODUCTION

IN THE COURSE OF ANALYSIS of various seaweeds for their content of trace elements, specimens of the same species in different localities were found with markedly different concentrations (Young and Langille, 1958). It was therefore desirable to compare the concentrations of such elements in the surrounding sea water with those in the plant.

While many routine determinations of salinity of the waters around the Atlantic Provinces of Canada have been made, no detailed chemical analysis was available. Such analyses in various parts of the world differ considerably, even in order of magnitude, for such elements as Ni, Mo, Mn, and Co. Few have been made at the same time as were those of Black and Mitchell (1952). We have therefore carried out a systematic analysis of major and many minor elements of the sea water in this area.

The data obtained are of interest in themselves since few systematic analyses of trace elements in sea water have been made, as noted above. They may also indicate micro-nutrients as possible ecological factors in the growth of algae. Thus in the neighbourhood of Sable Island appears to occur the most prolific growth of rockweeds in the world (MacFarlane, 1952). *Chondrus crispus* is very abundant

[1]Received for publication August 5, 1958.

J. FISH. RES. BD. CANADA, **16**(1), 1959.
Printed in Canada.

off Prince Edward Island and *Rhodymenia palmata* off Grand Manan Island. Seaweeds are sparsely distributed and not typically marine in the Bras d'Or Lakes of Cape Breton Island (Bell and MacFarlane, 1933).

EXPERIMENTAL

Samples of water were collected from the surface in polyethylene bottles of 2 Imp. gallon capacity from eight stations, during August and September, 1956. The exact positions are recorded in Table I. The temperature varied between 43 and

TABLE I. Area of collection and physical characteristics of the samples.

Sample	Locality	Latitude N	Longitude W	Sp. gr. (15.5°C)	pH
1	Bras d'Or Lakes East Bay, N.S.	46°	60°27′	1.016	7.98
2	Grand Manan, N.B.	44°47′	66°42′	1.023	7.62
3	W. Cape Sable, N.S. Seal Island	43°17′	66°	1.023	7.70
4	E. Cape Sable, N.S. Lockport	43°39′	64°45′	1.022	7.87
5	St. Andrews, N.B.	45°12′	67°	1.021	7.78
6	Halifax, N.S.	44°38′	63°35′	1.021	7.82
7	NE. Cape Breton, N.S. Scateri Islands	45°55′	59°45′	1.021	7.87
8	Malpeque Bay, P.E.I.	46°40′	63°40′	1.020	7.88

48°F but was not recorded for all samples. In some cases analysis was started within a few hours (No. 6) or days (No. 1, 5) while in others there was an interval of several weeks. The semi-permeability of polyethylene was not considered an important factor for the degree of accuracy involved in these determinations (Cox, 1954).

In the laboratory the whole sample was filtered with suction through Whatman No. 50 filter paper on a large Büchner funnel. A portion of the filtrate was evaporated to dryness on a steam bath in a current of warm air. The fluid was always alkaline during this operation. The crystalline residue was dried in an oven at 110°C for several hours. The dry mass was triturated in a mortar, weighed, and blended in a Fisher–Kendall mixer. Determinations of Co, Mo, Zn, Cu, Si, and I were made on this material.

METHODS

Specific gravity was determined by spindle at 15.5°C, pH by Beckman pH-meter, chlorinity by titration with standard silver nitrate, salinity by Knudsen's equation from the chlorinity. For other constituents the following methods were employed.

Arsenic, by a slightly modified Gutzeit procedure (A.O.A.C., 1950) at a sensitivity of 0.3 μg on the concentrate from 500 ml of sea water with standard strips made from 1% mercuric bromide. The sensitivity of the molybdenum blue method (Jacobs and Nagler, 1942), or that of Gorgy *et al.* (1948), at 1.5 μg, was insufficient even in a Coleman No. 14 spectrophotometer.

Boron, colorimetrically by the quinalizarin method of MacDougall and Biggs (1952).

Calcium, as oxalate by permanganate titration (A.P.H.A., 1955), confirmed by flame photometry.

Cobalt, molybdenum, and *zinc,* in an Applied Research Laboratories 1.5-m grating spectrograph by the method of Mitchell (1948) on a 20-g aliquot of the solids. After the requisite manipulation, the sample was arced on graphite electrodes of high purity for 40 sec at a current of 8 amp and at 32% transmission. The film was measured in an Applied Research Laboratories Projector-Comparator Densiometer. The wave lengths used were Co — 3453.5 Å, Zn — 3282.0 Å, and Mo — 3132.2 Å.

Copper, by the biquinoline method of Cheng and Bray (1953).

Fluorine, by the zirconium–alizarin method for water by distillation of 50-ml sample at 150°C and estimation in a Coleman Junior spectrophotometer at 540 $m\mu$ on first 100 ml of distillate (A.O.A.C., 1950; Rowley *et al.,* 1953).

Iodine, volumetrically on aqueous extract after alkaline fusion of 2 g of solid (A.O.A.C., 1950).

Magnesium, gravimetrically as pyrophosphate after removal of calcium and three precipitations as ammonium magnesium phosphate (Epperson, 1928).

Phosphate (inorganic), by the stannous chloride method of Harvey (1948) on 100 ml of sea water, in a micro-cuvette of 5-cm path length in a Coleman No. 14 spectrophotometer with red filter at 700 $m\mu$. The amount of salt in the water inhibited the development of colour in both the aminonaphthol-sulphonic acid and the ordinary stannous chloride methods. The method employed must be considered as only semi-quantitative at a sensitivity of about 0.5 μg.

Silicon, by the standard method of the A.O.A.C. (1950).

Sodium and *potassium,* by flame photometer directly on diluted sea water.

Sulphate, gravimetrically as barium sulphate (A.P.H.A., 1955).

RESULTS

The results are shown in Tables I, II and III. The ratios of several elements to chlorine, and the Ca/Mg ratio, are given in Table IV. The salinity and the concentrations of the major constituents of the sample of water (No. 1) from the Bras d'Or Lakes were low in comparison to open ocean waters. The ratios for this sample are all normal except for Na/Cl which is lower at 0.546 than the range in Table IV. The concentrations of minor constituents are, for the most part, comparable to those of the other localities and zinc is notably high. The peculiar nature of these salt-water lakes where there is little tidal motion and very limited access to the ocean must permit of great dilution by fresh water from their watershed.

The other samples (No. 2 to 8) show some interesting differences. The levels of chlorinity and salinity are low compared to those of ocean water but rise from

TABLE II. Concentrations of the major constituents (as grams per kilograms).

Sample	Salinity	Chlorinity	Na	K	Ca	Mg	SO_4	H_3BO_3
1	20.43	11.30	6.17	0.226	0.246	0.769	1.60	...
2	32.15	17.79	9.79	0.323	0.381	1.135	2.46	...
3	31.73	17.56	9.66	0.323	0.362	1.086	2.37	...
4	31.09	17.20	9.66	0.352	0.382	1.164	2.36	0.0249
5	31.33	17.34	9.67	0.353	0.385	1.197	2.41	0.0252
6	30.45	16.85	9.40	0.340	0.360	1.142	2.31	0.0235
7	30.41	16.83	9.40	0.353	0.379	1.161	2.35	0.0235
8	30.05	16.63	9.28	0.343	0.366	1.146	2.23	...
Av. (No. 2–8)	31.03	17.17	9.55	0.341	0.374	1.147	2.36	0.0243
Range	29 to 40*	16 to 22*	8.87 to 12.37*	0.30 to 0.47*	0.33 to 0.46*	0.95 to 1.28†	2.2 to 3.1*	0.008 to 0.030‡

*Harvey (1955); †Thompson and Wright (1930); ‡Igelsrud *et al.* (1938).

TABLE III. Concentrations of some minor constituents (as micrograms per litre).

Sample	F	As_2O_3	I	Co	Mo	Zn	Cu	Si	PO_4
1	600	1.0	20	0.65	8.2	10.9	14.1	91	15
2	1180	2.0	53	0.34	10.2	6.8	22.2	106	5
3	1080	1.8	6	0.66	6.3	6.6	13.2	87	5
4	1080	1.4	10	0.34	14.0	6.8	27.3	95	5
5	1120	2.0	25	0.65	5.9	6.5	21.3	76	13
6	1200	1.6	10	0.67	9.7	6.7	16.7	61	69
7	960	1.6	15	0.33	7.9	6.5	14.7	44	31
8	860	1.4	15	0.66	6.6	6.6	20.7	91	22
Range	1000 to 1400*	1.6 to 5*	9 to 75†	0.1 to <0.3‡	12 to 16*	9 to 21*	1 to 25*	10 to 1000*	3 to 180*

*Harvey (1955); †Thompson and Robertson (1932); ‡Black and Mitchell (1952).

TABLE IV. Ratios of mineral elements in samples 2 to 8.

	Range	Average	Previous values
Na/Cl	0.550 –0.562	0.556	0.555*
K/Cl	0.0180 –0.0210	0.0198	0.0202*
Ca/Cl	0.0206 –0.0225	0.0217	0.0218*
Mg/Cl	0.0619 –0.0690	0.0669	0.069†
SO_4/Cl	0.134 –0.140	0.138	0.140*
H_3BO_3/Cl	0.00139–0.00145	0.00142	0.00137*
Ca/Mg	0.316 –0.333	0.322	0.324†
F/Cl × 10^5	5.2 –7.1	6.2	7‡

*Harvey (1955); †Thompson and Wright (1930); ‡Thompson and Taylor (1933).

the lowest values near Prince Edward Island around the Nova Scotian coast southward to the Bay of Fundy. These results are merely confirmatory of previous observations (Hachey *et al.*, 1954).

The concentrations of sodium and potassium are relatively low for ocean waters. The contents of calcium and magnesium are within the usual ranges and remarkably constant. The sulphate was in the lower portion of the usual range and the average SO_4/Cl ratio (0.138) was slightly lower than that found by previous observers (Thompson *et al.*, 1927, 1931).

The boron content, expressed as boric acid, was of the same order of magnitude as in the Pacific Ocean (Igelsrud *et al.*, 1938; Miyaka, 1939). Due to shortage of material only samples 4 to 7 could be analyzed after it was found that analysis of the dry salt mixtures was unreliable for this element.

The content of inorganic phosphate was variable and may represent seasonal fluctuation. That of the sample of water from Halifax Harbour (No. 6) was higher than the others. The presence of water from the St. Lawrence River appeared to raise the concentrations in samples 7 and 8. Those from around the southern part of Nova Scotia at 5 μg/l were low.

Of the trace elements cobalt was notably higher and iodine somewhat lower than the few previous determinations in the literature. The levels of molybdenum agreed with those of Black and Mitchell (1952) in contrast to the earlier and much lower figures in the literature. Copper, zinc, and silicon were in agreement with recognized levels in other parts of the world.

Nickel was present in all of our samples but was not determined quantitatively. Vanadium could not be detected by the procedure of Mitchell (1948).

The range in concentration of arsenic as arsenite, expressed as As_2O_3, was 1 to 2 μg/l. Gorgy *et al.* (1948) claim that arsenite constitutes 60% of the total arsenic in surface sea water. These authors found 15 to 22 μg/l as arsenite As in water of the Pacific Ocean. This is equivalent to 20 to 29 μg/l as As_2O_3. These results are very high as compared to our own and those of Ishibashi *et al.* (1951) at 3 to 6 μg/l (Pacific Ocean), of Smales and Pate (1952) at 1.6 to 5 μg/l, and of Harvey (1955) at 2.4 to 3.1 μg/l (English Channel).

The fluorine content was relatively constant at about 1.1 ppm. This is in agreement with the results of Thompson and Taylor (1933) at 1.2 ppm and of Miyake (1939) at 1.3 ppm.

The average ratios of some mineral elements, especially to chlorine, in Table IV, show little variation from previous values obtained in other parts of the world, and this appears to indicate that they apply to off-shore waters of comparatively low salinity.

ACKNOWLEDGMENTS

The authors wish to thank Mr H. J. McLellan and Dr L. Lauzier of the Atlantic Oceanographic Group of the Fisheries Research Board of Canada for the collection of most of the samples of sea water. They are indebted to the Nova Scotia Research Foundation for the use of the spectrograph, and to Mr K. S. MacLean, Mr F. G. Mason, and Miss J. Macpherson for technical assistance.

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The Action of *Pseudomonas* on Fish Muscle: 3. Identification of Organisms Producing Fruity and Oniony Odours[1, 2]

By C. H. Castell, Maxine F. Greenough and Jacqueline Dale

Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

Bacteria capable of producing fruity and onion-like odours have been isolated from Atlantic cod and haddock fillets that had developed off-odours of this type. These organisms have been identified as being non-proteolytic strains of *Pseudomonas fragi*.

Compared to other fish-spoiling bacteria, including cultures of *Serratia, Proteus, Achromobacter,* and green pigmented *Pseudomonas, Ps. fragi* is quite sensitive to the bacteriostatic action of antibiotics of the tetracycline group.

Among the off-odours that frequently develop during the early stages in the spoilage of chilled fillets are those that have been described as "sweet" and "fruity". It has been shown by Castell and Greenough (1957) that bacteria capable of producing these odours can be isolated from fresh and spoiling fillets and that they belong to the genus *Pseudomonas.*

This paper deals with a group of twenty cultures of *Pseudomonas* that have been isolated from Canadian Atlantic fish, which produce distinct fruity and/or oniony odours when grown as pure cultures on fish muscle. The purpose has been to identify and describe these organisms.

Cultural Methods and Classification

Unless otherwise mentioned, the methods used for culturing and identifying these bacteria were either standard procedures or those given in the first paper in this series (Castell and Greenough, 1957).

A summary of the ordinary cultural characteristics of these organisms is given below. In general the twenty cultures showed a remarkable uniformity in colony formation, staining reactions, carbohydrate fermentations and in their inability to decompose protein and reduce nitrates to nitrites, etc. The variations that did occur were in their ability to hydrolyze fats and produce nitrite from hydroxylamine.

The observed characteristics of these cultures indicate that they are very similar to *Ps. fragi* (Eichholz) Huss. Emend. (Hussong *et al.,* 1937). It is of interest to note that this organism is characterized as producing a "sweet, ester-like odour resembling that of the flower of the May apple".

The cultures isolated from the fillets differ from the description of *Ps. fragi* given in Bergey's Manual (1948) in producing acid from maltose and in some cases from sucrose and glycerol, and in not liquefying gelatin. These differences

[1]Received for publication June 3, 1958.
[2]The previous paper in this series appeared in this Journal 15(4): 771–774, 1957.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

are relatively unimportant. Hussong *et al.* (1937) point out the extreme variability in different strains of *Ps. fragi*. They isolated three distinct types of colonies that differed in their biochemical reactions and particularly in their proteolytic and lipolytic activities. Munoz *et al.* (1949) in their study of the species of the genus *Pseudomonas* describe *Ps. fragi* as producing acid from maltose and sucrose, and being unable to liquefy gelatin.

Growth and Reaction on Single Sources of Carbon

Many authors have pointed out that fermentation studies made with members of the *Pseudomonas* often give unsatisfactory and sometimes contradictory results. Considerable difficulty was encountered in this work with cultures of *Ps. fragi* isolated from fish, in determining their reaction in various sugar broths. In some instances an acid reaction did not develop until the cultures had been incubated 18 to 20 days at 20°C, and occasionally after another couple of days they again became alkaline. This phenomenon has been observed before, with the result that some workers specify two different incubation periods (usually 5 and 20 days) for recording the action of *Pseudomonas* cultures on certain carbohydrates. It is probable that the rapid production of ammonia from the nitrogenous constituents, together with their relatively weak action on the carbohydrates, may account for this. To overcome this difficulty, substrates have been recommended in which the carbohydrates to be tested are used as the sole source of carbon, and the nitrogen is supplied in the form of an ammonium salt (Seleen and Stark, 1943; Liu, 1953). For this work the following basic medium was used:

Sodium ammonium phosphate	1.5 g
Magnesium sulphate	1.0 g
Monopotassium phosphate	1.0 g
Agar	10.0 g
Carbon source	3.0 g
Water	1000 ml.

In addition to fermentable carbohydrates, the sodium salts of several organic acids were also tested. In these media all twenty cultures gave similar results, which were as follow:

(a) No growth occurred with lactose, raffinose, salicin, inulin, starch, or dulcite.

(b) Growth, an acid reaction, but no gas formation, occurred with dextrose, maltose, galactose, levulose, sucrose, xylose, arabinose, cellobiose, mannitol, rhamnose, glycerol, and *d*-mannose.

(c) Growth with an alkaline reaction occurred with the sodium salts of citric, lactic, acetic, succinic, formic and benzoic acids. With sodium formate and sodium benzoate the growth was slower and less abundant than with the other salts.

(d) No growth occurred with the sodium salts of oxalic and salicylic acids in the concentrations used.

Under these conditions, the action of the organisms on the carbohydrates was quite definite. By comparing these reactions with those shown below for the ordinary

sugar broths, it can be seen that many of the carbohydrates not utilized in the ordinary sugar broths are readily attacked when used as the only source of carbon and in the absence of complex nitrogenous materials.

The characteristics for these twenty *Pseudomonas,* using standard methods and culture media, are as follow:

Source: fresh and spoiling fillets of cod, haddock, flounder, and halibut.
Origin: unknown, but probably soil, fresh water, and freshwater ice.
Small rods, motile with polar flagella; gram negative; non-spore-forming.
Agar colonies: round, convex, glistening; water-soluble green or blue-green pigments not formed; strongly cytochrome oxidase positive.
Agar slants: abundant growth with a tendency to spread; smooth white glistening surface; smooth to finely undulated edges; growth denser and slightly elevated at edges of colony.
Fats (butterfat, triolein, tributyrin) hydrolyzed by 15 out of 20 cultures.
Proteins (casein, gluten, gliadin, zein) not hydrolyzed.
Gelatin not liquefied.
Ammonia produced from peptone and asparagine.
Nitrates not reduced to nitrites or nitrogen.
Nitrites reduced by only one culture.
Nitrites formed from hydroxylamine by 17 cultures.
Indole not formed.
Acetylmethylcarbinol not formed.
Litmus milk: generally acid ring followed by acid coagulation and reduction of indicator; some turn alkaline without coagulation; milk never peptonized.
BCP milk: generally alkaline at first, followed by acidity and coagulation; a few cultures remain alkaline up to 30 days.
Trimethylamine not formed from either trimethylamine oxide or choline.
Cellulose, starch, and dextrin not hydrolyzed.
Acid but not gas from dextrose and galactose; acid but not gas from maltose after lengthy incubation (5 to 30 days).
After lengthy incubation (5 to 30 days) some cultures produce acid but not gas from sucrose, rhamnose, arabinose, and glycerol.
Neither acid nor gas from lactose, levulose, mannose, cellobiose, raffinose, inositol, *d*-mannitol, salicin, inulin, or ethanol.
Hydrogen sulphide either not produced or only in traces from tryptone, cystine, and methionine.
Urea not decomposed to ammonia.
Temperature range: grows slowly at −1° to +1°C; fair at 5° to 10°C; rapidly at 15° to 25°C; no growth at 37°C and above.
Lower pH limits on NaOH–phosphate buffered beef extract agar: none grew at pH 4.8 and below; many grew slowly at pH 5.1; all grew slowly at pH 5.4.

pH Tolerance for Growth

The pH range that permitted growth varied with the substrate used. On nutrient agar, adjusted with buffer solutions, nine of the twenty cultures grew very slowly at pH 5.1; all grew, but not abundantly in the range 5.4 to 6.6; as the pH values were raised to 7.0 from 6.6 there was a noticeable increase in the growth of all cultures. Between pH 7.0 and 8.8 all cultures grew well. When certain amino acids were used as sole nitrogen sources, no observable growth occurred at pH 6.5 and below; between 6.7 and 6.9 growth was retarded and in the range of 7.0 to 8.0 growth was abundant.

Salt Tolerance

In broth cultures these organisms grew well in the presence of 1 to 3% sodium chloride; 4 to 6% salt retarded growth for several days while 7% or more entirely inhibited growth.

The substitution of sea water for tap water did not enhance the ability of these organisms to grow in any of the media that were tested. There was nothing to indicate that these organisms are of marine origin. Similar cultures have been isolated from freshwater ice, soil, and fresh water supplies of fish plants in Nova Scotia.

Sensitivity to Antibiotics

During the grading and organoleptic examination of fillets it has frequently been observed that those treated with certain antibiotics rarely develop fruity odours. This is in sharp contrast to fillets treated with sodium nitrite, where the fruity stage is often prolonged and accentuated when compared with the controls (Castell and Gunnarsson, 1956; Castell and Greenough, 1957). These observations suggest that the antibiotics may have a selective action on the spoilage organisms.

Preliminary tests with six cultures of *Ps. fragi* showed that they were very sensitive to chlortetracycline (CTC) and tetracycline; moderately sensitive to oxytetracycline (OTC) and streptomycin; slightly sensitive to chlormycetin, polymyxin B and neomycin; and relatively insensitive to penicillin, bacitracin, dehydrostreptomycin, erythromycin, viomycin, and magnamycin. These results are somewhat similar to those in the early work of Tarr *et al.* (1952) in which they showed that CTC, OTC, and chloramphenicol were the most effective in retarding the growth of bacteria in fish muscle.

For more detailed study, further tests were made with the two broad-spectrum antibiotics which are now legal for use on fresh fish in Canada: CTC and OTC. This was done by a series of tests in which measured amounts of these antibiotics were added to nutrient broth and then inoculated with the organisms to be tested. The inoculum consisted of one drop of a 24-hour broth culture into 10 ml of the nutrient broth containing the antibiotic.

The initial concentration of the antibiotic necessary to inhibit growth increases as the incubation period is prolonged, probably the result of a partial breakdown of the antibiotics.

Table I gives the minimum concentrations of CTC and OTC that inhibited the growth of twelve fish-spoiling organisms for 1, 2, and 4 days in the nutrient broth at 25°C. Four similar sets of tests were made with the same cultures. In each case the results were approximately the same. It can be seen that less of each of these antibiotics was required to prevent the growth of *Ps. fragi* and *Ps. perolens* than for any of the other organisms. *Serratia marcescens* and *Proteus vulgaris* are relatively insensitive to these antibiotics.

In addition to the achromogenic *Pseudomonas*, large numbers of green and blue-green *Pseudomonas* are frequently encountered on spoiling fish muscle. The majority are strongly proteolytic and rapidly produce obnoxious odours when grown on fish muscle. Table II compares the sensitivity to four antibiotics of six typical proteolytic, green-fluorescent cultures and six cultures of *Ps. fragi*. The results show that the latter were the most sensitive to each of these antibiotics.

TABLE I. Minimum initial concentrations (using 1, 2, 3, 4, 5, 7, 10, 20, 30, 50, 100, 150, 200 ppm) of chlortetracycline (CTC) and oxytetracycline (OTC) that prevented growth of twelve fish-spoiling organisms in nutrient broth at 25°C.

Organism	Inhibition concentrations in ppm					
	CTC			OTC		
	1 day	2 days	4 days	1 day	2 days	4 days
Serratia marcescens	100	150	250	100	150	200
Proteus vulgaris	50	100	150	40	100	150
Pseudomonas putrefaciens	20	20	30	20	20	20
Pseudomonas fluorescens	3	20	20	4	10	20
Flavobacter marinum	3	20	20	4	10	20
Achromobacter No. 176	3	7	20	4	4	10
Aerobacter aerogenes	1	2	30	1	3	30
Flavobacter solare	1	5	5	1	7	10
Flavobacter No. Y17	1	10	20	1	10	10
Pseudomonas fragi 4A	1	1	3	1	1	1
Pseudomonas fragi B1	1	1	3	1	2	2
Pseudomonas perolens 220	1	1	3	1	1	2

TABLE II. Minimum initial concentrations of polycycline, streptomycin sulphate, chlortetracycline hydrochloride (CTC) and oxytetracycline hydrochloride (OTC) required to prevent growth of six cultures of *Ps. fragi* and six cultures of green-pigmented proteolytic *Pseudomonas* (G-p *Ps.*) in the nutrient broth at 25°C at 24, 48, and 72 hours. The initial concentrations of these antibiotics that were used were 1, 2, 3, 4, 5, 7, 10, 20, 30, 40, and 50 ppm.

Organism	Polycycline			Streptomycin			CTC			OTC		
	24	48	72	24	48	72	24	48	72	24	48	72
	hours			*hours*			*hours*			*hours*		
Ps. fragi 4A	1	2	2	5	7	7	1	1	2	2	2	2
" B1	1	2	2	10	10	10	1	1	2	2	3	3
" 28	1	2	2	5	10	10	1	2	2	2	2	2
" F6	1	1	1	4	7	7	1	1	1	1	1	1
" 24	1	2	2	5	5	5	2	2	2	1	1	2
" 31	1	2	2	4	10	10	1	2	2	1	1	2
G-p *Ps.* 236	2	7	10	20	30	30	2	5	10	5	7	10
" 84	3	7	10	10	10	20	3	5	10	7	10	20
" 90	5	10	30	10	30	50	3	7	10	2	7	7
" 78	2	7	10	7	20	20	2	5	7	2	7	7
" 210	4	10	10	10	40	50	2	5	10	4	10	10
" 224	3	7	10	20	20	50	2	5	7	4	5	10

INHIBITION OF *Ps. fragi* BY SODIUM NITRITE

Sodium nitrite in concentrations ranging from 10 to 10,000 ppm was added to nutrient broth buffered at pH 6.5, 7.0, 7.5, and 8.0. These media were inoculated with four strains of *Ps. fragi* and incubated at 25°C. The concentrations of sodium nitrite were: 10, 50, 100, 500, 1000, 1500, 2000, 3000, 4000, 5000, and 10,000 ppm. Table III shows the maximum of these concentrations permitting

TABLE III. Concentrations of sodium nitrite that inhibited growth of *Ps. fragi* in nutrient broth solutions buffered at pH 6.5, 7.0, 7.5, and 8.0 during incubation for 1, 2, and 3 days, at 25°C.

pH	Concentrations of nitrite in ppm		
	1 day	2 days	3 days
6.5	100	500	500
7.0	1,500	1,500	2,000
7.5	10,000	10,000	10,000
8.0	10,000	10,000	10,000

growth up to 72 hours incubation. There was no further change when the incubation period was prolonged to 18 days.

DISCUSSION

These results would indicate that *Ps. fragi,* known to be one of the causes of the development of fruity odours in dairy products, plays a similar role in the spoilage of chilled fish muscle.

In a study of the distribution of *Ps. fragi* in the United States, Morrison and Hammer (1941) found it to be widely distributed in soils and in dairy plant water supplies. In soil samples it was more frequently found in the eastern than in the western United States. They failed to find it in soils of southern United States (Kentucky) during the warm summer period. This might be expected as *Ps. fragi* is definitely a psychrophilic species.

Ps. fragi has not been isolated in this laboratory from sea water or from fish slime at the time the fish comes from the water. It has been found occasionally on the surface of gutted Atlantic cod and haddock after they had been stored in ice for several days on the trawlers. Nothing that has been observed about its distribution or its cultural characteristics would indicate that it is of marine origin.

It has been isolated very frequently from the muscle of Atlantic cod, haddock, flounder and halibut, after they had been stored as fillets or steaks. It is readily isolated from fish muscle that has developed typical fruity odours. It grows well on protein-rich media, such as peptones, meat extracts, or protein hydrolysates, and in the absence of sugars or other carbohydrates. It grows slowly and produces typical fruity odours on fish muscle and other protein-rich substrates at 0°C and grows rapidly at 5 to 10°C. Except in the case of very heavy inocula, it does not grow at 37°C.

It would seem probable that *Ps. fragi* gets onto fish mainly through the ice and water used for their preservation or cleaning, or from plant or boat surfaces that have been previously contaminated by these materials. Moist, cool surfaces, especially if covered with fish juices, are ideal breeding places for these organisms.

It is interesting to note that all the cultures of *Ps. fragi* isolated from fish have been non-proteolytic, while dairy bacteriologists (Hussong *et al.,* 1937) found many strains that were able to decompose proteins. In an early review of the micro-organisms producing various types of sweet, fruity, and ester-like aromas, Omelianski (1923) describes both proteolytic and non-proteolytic species. This might be the result of the method by which the cultures from the fish have been selected. Over a period of years a collection has been made of those organisms isolated from fish producing typical strong fruity odours. It could be that proteolytic strains have been missed because in their case the ester-like odours have been quickly replaced by the odour of some of the more obnoxious end-products of protein decomposition.

It is also interesting to note that these organisms which play a considerable part in the early stages of fish muscle spoilage are relatively inactive, as judged by biochemical reactions used in their identification. They ferment few sugars; they do not hydrolyze proteins, reduce trimethylamine oxide, decompose urea, reduce

nitrate, form indole, or produce hydrogen sulphide. Although some strains hydrolyze fats, this is not involved in the production of fruity odours, which are developed just as readily by the non-lipolytic strains.

SUMMARY

Twenty cultures of bacteria that produce typical fruity odours on fish muscle have all been identified as non-proteolytic strains of *Ps. fragi*. They probably originate in soil and fresh water and get onto the fish through ice, plant water supplies, and contaminated surfaces in the plant and on the boats.

These organisms are relatively sensitive to the broad-spectrum antibiotics; they are relatively insensitive to small concentrations of sodium nitrite at pH values of 6.5 and 7.0.

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The Action of *Pseudomonas* on Fish Muscle: 4. Relation between Substrate Composition and the Development of Odours by *Pseudomonas fragi*[1, 2]

By C. H. Castell and Maxine F. Greenough

Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

The characteristic fruity and onion-like odours produced by *Ps. fragi* are the result of these organisms acting on substrates containing partially hydrolyzed proteins. It has been shown that the immediate protein derivatives from which the bacteria produce the fruity odours are mono-amino monocarboxylic acids. The fruity smelling compounds are probably volatile esters formed after bacterial deamination of these amino acids.

Under some conditions these organisms are able to produce various types of sour-onion and garlic-like odours. It has been found that these odours are also produced through the action of the bacteria on amino acids, but are not restricted to the mono-amino monocarboxylic group. Nothing is yet known of the mechanism by which these onion-like odours are formed.

Fruity and oniony odours are only two of many different odours produced by the action of these organisms on amino acids. Among others, they are also able to produce odours suggesting cabbage, sour cabbage and rotten vegetables from methionine; cooked egg yolk, and hydrogen sulphide-like odours from cysteine, cystine, and glutathione; and various sour vegetable-like odours from other amino acids.

INTRODUCTION

This fourth paper in this series dealing with the bacteria responsible for the odours that develop during the earlier stages of spoilage in fish muscle, more particularly in chilled fillets of cod and haddock, deals further with the same group of twenty cultures that produce distinct fruity and/or oniony odours on fish muscle and which have been identified previously as being *Ps. fragi* (Castell, Greenough and Dale, 1959). The purpose of the work described here has been to determine under what conditions these particular odours are produced and from what components of the muscle they arise.

Identification of Odours

The description and identification of particular odours and the estimation of their intensity must necessarily be very subjective. It requires considerable skill and training. The persons who have judged odours in this work have not only had several years of experience but have also come to a common agreement on the terms to be used in describing each particular type of odour.

The terms "fruity", "oniony", and "vegetable-like" have been used in a generic sense. Odours classified as "fruity" include various ester-like odours suggesting pineapple, May apple, strawberry, melon, etc. "Oniony" odour includes those suggesting fresh onion or garlic, but more often sour and spoiling onions. The

[1]Received for publication June 3, 1958.

[2]Part 3 of this series appears on pp. 13–19 of this issue of this Journal.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

term "vegetable-like" is the most comprehensive and has been used for a variety of odours suggesting fresh and spoiling turnips, cabbage, and cauliflower. This type of odour frequently developed into a disagreeable sour odour that the judges identified with that coming from a kitchen drain pipe.

Most frequently the development of odours has been observed and followed by enclosing inoculated substrates in screw-capped bottles, which were periodically examined by removing the cap and noting the odours when the mouth of the bottle was held close to the nostrils. Experience demonstrated that unless appropriate intervals of time were permitted between smellings the ability to identify odours decreased rapidly.

PRODUCTION OF ODOURS BY *Ps. fragi*

Typical Odours on Complex Substrates

Although characteristic ester-like odours suggestive of various fruits are frequently produced by these cultures of *Ps. fragi*, it should be clearly understood that they are not the only odours produced, and on many different substrates they are not produced at all. Frequently when the fruity odours are produced, they are one stage in a succession of different odours. Whether the fruity odours are predominant, and what other odours accompany or follow them, seems to depend mainly upon the composition of the substrate.

In order to give a general idea of the odour-producing characteristics of these particular cultures the results of several sets of observations will be given. The first of these is summarized in Table I, which shows the odours observed after each of the twenty cultures had been growing for 48 hours on skim-milk agar, cod muscle, and a tryptone–sulphite–cystine agar (used for determining hydrogen sulphide production). It is seen that the tendency for the cultures to produce oniony rather than fruity odours becomes greater as the substrate is changed from

Table I. Predominating odours produced by twenty cultures of *Pseudomonas* inoculated onto the surface of three different substrates and examined after 48 hr at 20°C.

Culture No.	Skim-milk agar	Cod muscle	Tryptone–sulphite–cystine agar
4	fruity	fruity	fruity + oniony
20	fruity	fruity	oniony
28	musty + fruity	fruity	oniony
31	musty	sweet and vegetable	oniony
B–1	fruity	fruity	oniony + sour
Ps. fr.	fruity	fruity	oniony
2	musty + sweet	sweet	oniony
3	musty + sweet	sweet	oniony
5	musty	sweet + fruity	oniony
22	musty + fruity	fruity + sour	oniony
23	musty	fruity + oniony	oniony
29	musty	fruity	oniony
30	musty	fruity + oniony	oniony
C–2	musty	fruity	oniony
C–3	musty + sweet	fruity + sour	oniony
6	musty	fruity	oniony
24	musty	oniony + sour	oniony + fruity
F–4	musty	fruity + oniony	oniony
F–5	musty	fruity + oniony	oniony
F–6	musty + fruity	fruity + oniony	oniony

milk to fish to tryptone–sulphite–cystine agar. These observations were all made after 48 hours at 20°C, and are of interest for showing the effect of the difference in substrate on the odours produced. A better picture can be obtained by comparing a series of observations made at several successive periods. An example of this is given in Table II, which shows the observations when ten of these cultures were inoculated onto both sterile cod muscle and nutrient agar and examined after incubation periods of 1, 2, 3, and 6 days. This table indicates the change in odours as the incubation period is extended and also that the later odours from the fish are not the same as those from the nutrient agar.

The observations recorded in these two Tables, although somewhat condensed, are quite typical of the results obtained with these cultures. From these preliminary observations it is seen that from the substrates used, these cultures produce both fruity and oniony odours; that the odour or combination of odours at any particular time is apparently determined by the nature of the substrate, the extent of bacterial action that has occurred, and the culture that has been used. The fruity odours usually, but not invariably, precede the oniony odours when both occur from the activity of one culture. Some cultures (No. 4, 20, 28, 31, B1, and *Ps. fr.*) tend to produce more pronounced and prolonged fruity odours on skim milk, fish muscle, and nutrient agar, while others (No. 6, 24, F4, F5, and F6) rapidly develop oniony odours. Although this difference appears to be mostly a matter of degree, it has been found convenient to speak of the former group as the "fruity cultures" and the latter as the "oniony cultures". Others would appear to be intermediate between these groups.

TABLE II. Predominating odours produced by ten cultures of *Pseudomonas* on heat-sterilized cod muscle, and on nutrient agar when held at 20°C, and examined after successive periods of incubation.

Culture No.	Incubation periods			
	24 hours	48 hours	72 hours	6 days
Substrate: fish muscle				
4	fruity	fruity	fruity + sour	NH_3 + trace of sweet
20	fruity	fruity	fruity + sour	NH_3 + sweet
28	fruity	fruity	fruity + trace putrid	NH_3 + sweet fruity
C–3	fruity	fruity + trace putrid	fruity + trace putrid	NH_3
29	fruity	fruity	fruity	NH_3 + sweet
2	fruity	fruity + oniony	sour oniony	sweet fruity
6	fruity	fruity + oniony	sour oniony	NH_3 + putrid
F–4	fruity + oniony	fruity + oniony	sour oniony	NH_3 + putrid
F–5	fruity + oniony	fruity + oniony	sour oniony	NH_3 + putrid
F–6	fruity + oniony	fruity + oniony	sour oniony	NH_3 + putrid
Substrate: nutrient agar				
4	fruity	fruity	sour fruity	sweet
20	fruity	fruity	fruity	sweet
28	fruity	fruity	fruity sweet	sweet
C–3	fruity	fruity + oniony	sour vegetable-like	sour vegetables
29	sour fruity	fruity + oniony	oniony	sour vegetables
2	oniony	oniony	sour vegetable-like	sour oniony
6	sour fruity	oniony + fruity	oniony	sour oniony
F–4	oniony	oniony	oniony + trace of acetic acid	oniony + trace of acetic acid
F–5	oniony	oniony	oniony	sweet
F–6	oniony + fruity	oniony	oniony	oniony + musty

These tests were all made with relatively heavy inocula and incubated at 20°C. This, of course, is quite different from the conditions under which these organisms normally grow on chilled fish under commercial conditions where initial contamination is very much less and the temperature is usually closer to 0°C. The development and succession of odours is very much slower under the latter conditions.

Relation between Composition of Substrate and Odour Production

Many different materials were tested as substrates with these twenty cultures of *Ps. fragi* and records were kept of the odours that developed. It soon became apparent that nitrogenous materials, rather than fats, carbohydrates or inorganic compounds, were acted upon by these organisms to produce the fruity and oniony odours. The first tests were made with complex protein materials ordinarily used for culturing bacteria, including peptone, tryptone, beef extract, yeast extract, veal liver, skim milk, gelatin, and liver infusion. This was followed by tests with muscle from various types of fish, protein hydrolysates, individual amino acids, various amines, and simpler compounds such as urea, nitrates, nitrites, and ammonia.

Tabulation of these observations for the twenty cultures produced a very ponderous table which has not been included here. Some of the complex nitrogenous materials produced strong fruity odours. These included skim milk, peptone, tryptone, yeast extract, fish muscle, and hydrolysates of lactalbumin and casein. The principal point of interest was the odour production on media with amino acids.

In some cases the tests were made by inoculating an agar medium containing only 1% of a single amino acid and no additional salts or carbon compounds. With some of the amino acids, hydrogen ion concentration was too high to permit growth, or growth on the single compound was very retarded. Much more satisfactory results were obtained by adding 1% of the amino acid to basal agar medium containing: magnesium sulphate, 0.1%; dipotassium phosphate, 0.1%; sodium chloride, 0.5%; and either dextrose or sodium acetate, 0.2%. Where necessary the pH values were adjusted to bring them into the range of 6.7 to 7.0 after autoclaving.

Several of the uninoculated, amino-acid-containing, autoclaved media had very distinct odours. This was particularly so with the leucines (odours of amyl acetate and amyl alcohol), phenyl alanine (a fragrant odour suggesting hyacinths in bloom) and methionine (boiled potatoes). When these particular media were inoculated, their initial characteristic odours either disappeared or were masked as the bacterial activity increased.

The odours from two cultures (fruity culture 4A and oniony culture F6) are summarized in Tables III and IV for twenty amino acids or related compounds. These are quite representative of the odours produced in a whole series of tests using the twenty cultures.

As might be expected there were considerable differences in the ability of various amino acids to support growth, when used as sole sources of nitrogen.

TABLE III. Predominating odours produced by fruity culture 4A growing on agar media containing 1% of various amino acids and closely related compounds after 6 to 14 days incubation at 45°F.

Amino acid	Growth**	Predominating odours*					
		Uninoculated controls	6 days	7 days	8 days	10 days	14 days
Glycine	+	–	–	slightly sweet	fruity	fruity	fruity
DL–α–Alanine	+++	sour + scorched paper	vegetable	sweet fruity	sour fruity	–	NH_3 + sweet
β–Alanine	+	–	–	–	musty, earthy	musty + fruity	sour
DL–Valine	++	amyl acetate	–	slightly fruity	fragrant, sweet	slightly sweet	–
DL–Leucine	++	amyl alcohol	fragrant	sour fruity	fruity	sour	sour fruity
DL–Isoleucine	+	amyl acetate	fruity	fruity	sweet	sweet	sweet fruity
DL–Norleucine	++	amyl alcohol	–	fragrant	sweet + ?	sweet + ?	sweet + ?
DL–Serine	+++	–	fruity + sour	sour fruity	fruity	Sweet + NH_3	NH_3 + fruity
DL–Threonine	nil	–	–	–	–	–	–
DL–Phenylalanine	++	hyacinth	fruity + fragrant	strong hyacinth	hyacinth +	hyacinth +	fruity + ?
L–Hydroxyproline	+++	trace of oniony	sour vegetable	sweet + ?	fruity	fruity + oniony	sour veget.
L–Cystine	+	–	–	–	–	–	–
DL–Methionine	+	boiled potato	–	boiled potato	boiled potato	boiled potato	boiled potato
L–Arginine	nil	–	–	–	–	–	–
β–Aminobutyric acid	nil	scorched paper	–	–	–	–	–
DL–Asparagine	++	–	sour vegetable	sl. sour veget.	sour fruity	fruity	NH_3 + sweet
Creatine	++	–	rotten veget.	rotten turnip	faecal sweet	green oniony	slightly veget.
Betaine	+	scorched paper	sl. sour veget.	sweet fruity	fruity	sour vegetable	cooked veget.
Taurine	++	–	slightly fruity	–	slightly sweet	sweet	–
Xanthine	+	–	–	sour vegetable	rotten veget.	–	–

*– Indicates no observable odour.
**+ Indicates poor retarded growth, ++ good growth, +++ abundant growth.

TABLE IV. Predominating odours produced by oniony culture F–6 growing on agar media containing 1% of various amino acids and closely related compounds after 6 to 14 days incubation at 45°F.

Amino acid	Predominating odours* (for odours from control plates, see Table III)					
	Growth**	6 days	7 days	8 days	10 days	14 days
Glycine	+++	–	sweet	sour oniony	NH_3	NH_3
DL–α–Alanine	+++	rotten – vegetable + oniony	sour oniony	sour oniony	sour oniony	sweet pickle
β–Alanine	+	–	–	musty	musty + green potato	green oniony
Valine	++	fruity + oniony	sour oniony	sour oniony	oniony + kerosene	oniony
DL–Leucine	++	–	sour	sour	sour	oniony
Isoleucine	+	–	sweet	–	green oniony	fruity
Norleucine	+	–	–	–	sweet	–
DL–Serine	+++	sour fruit + oniony	sour veget. + oniony	sour oniony	musty + sour	NH_3
Threonine	nil?	–	–	–	–	sweet + oniony
Phenylalanine	+++	–	–	sour oniony	rotting flowers	oniony
Hydroxyproline	+++	sour oniony	sour oniony	sour oniony	?	NH_3 + oniony
L–Cystine	nil?	–	–	–	–	–
Methionine	+	–	boiled potato	boiled potato	potato + rotten turnip	boiled potato
Arginine	nil	–	–	–	–	–
Aminobutyric acid	+	–	–	–	cooked veget.	fruity
DL–Asparagine	+++	sour veget.	rotten veget.	sour oniony	NH_3 + ?	NH_3 + oniony
Creatine	nil	–	slightly sweet?	–	–	–
Betaine	+	oniony	oniony	sour oniony	sour oniony	NH_3 + oniony
Taurine	++	slightly fruity	–	sour oniony	slightly sweet	sweet + ?
Xanthine	+	–	rotten veget.	sl. musty + sl. faecal	–	–

*– Indicates no observable odour.
**+ Indicates retarded growth, ++ good growth, +++ abundant growth.

Some idea of the odours produced from these compounds by the bacteria was obtained by adding them to a more complete medium and noting any additional odours that were produced. Under these conditions, methionine, for example, produced a strong cabbage odour which later became like sour cabbage and then very rotten vegetables. With some cultures, cystine in a more complete medium produced an odour resembling cooked egg yolk and then an odour of hydrogen sulphide.

Our chief interest, however, was to determine which of the amino acids produced fruity and/or oniony odours when acted upon by these cultures. This has been summarized in Table V. Fruity odours were produced only from acids of the mono-amino monocarboxylic group. In contrast to this the oniony odours were produced from a wide range of amino acids including some of the mono-amino, di-amino, and heterocyclic types.

Certain other compounds, closely related to the amino acids, also produced fruity odours (asparagine, betaine), while others did not (creatine) or only in traces (taurine). Oniony odours were produced at some stage in the action of the oniony cultures on each of these compounds.

Various amines were also tested. As sole sources of nitrogen, most (trimethylamine oxide, trimethylamine, dimethylamine, monomethylamine, di- and triethylamine) did not support the growth of any of these cultures. Ethanolamine supported moderate growth. None produced oniony or fruity odours.

Fruity and oniony odours were not produced from choline, glucosamine, histamine, pyridine, piperidine, piperazine, urea, nitrate, nitrite, or ammonia.

TABLE V. Production of fruity odours and oniony odours from amino acids by *Pseudomonas* cultures. (– Indicates no observable odours produced; N.G. signifies the amino acid not only failed to support growth of these organisms but also inhibited growth in concentrations of 1%, when added to a more complete medium.)

Common name	Systematic name	Fruity	Oniony
Glycine	amino ethanoic acid	+	+
α–Alanine	2–amino propanoic acid	+	+
Valine	2–amino–3–methyl butanoic acid	+	+
Leucine	2–amino–4–methyl pentanoic acid	+	+
Isoleucine	2–amino–3–methyl pentanoic acid	+	+
Norleucine	2–amino hexanoic acid	N.G.	N.G.
Serine	2–amino–3–hydroxy propanoic acid	+	+
Threonine	2–amino–3–hydroxy butanoic acid	+	+
Phenylalanine	2–amino–3–phenyl propanoic acid	+	+
Tyrosine	2–amino–3–p–hydroxyphenyl propanoic acid	–	+
Thyroxine	3 [3, 5–diiodo–4–hydroxy–phenoxy) –3, 5–diiodophenyl] –3–amino propanoic acid	N.G.	N.G.
Tryptophan	2–amino–3–indole propanoic acid	N.G.	N.G.
Proline	pyrrolidine–2–carboxylic acid	–	+
Hydroxyproline	4–hydroxypyrrolodine–2–carboxylic acid	–	+
Aminobutyric acid	2–amino butanoic acid	N.G.	N.G.
Aspartic acid	2–amino butane dioic acid	–	+
Glutamic acid	2–amino pentane dioic acid	–	+
Cystine	3, 3′–dithiobis (2–amino propanoic acid)	–	–
Homocystine	3, 3′–dithiobis (2–amino butanoic acid)	N.G.	N.G.
Methionine	2–amino–4–methylthio butanoic acid	–	–
Ornithine	2, 5–diamino pentanoic acid	–	+
Lysine	2, 6–diamino hexanoic acid	–	+
Arginine	2–amino–5–guanido pentanoic acid	–	+
Histidine	2–amino–3 (5–imidazole) propanoic acid	–	+

Effect of Added Carbohydrates

In many cases the addition of small amounts of carbohydrates to a substrate resulted in better and faster growth. In a few instances the addition of a carbohydrate to the nitrogenous medium modified the fruity odours, so that the judges used terms such as "sharp fruity" or "sour fruity", rather than "fruity". But in none of the many tests made could it be demonstrated that the action of the organism on a carbohydrate resulted in the development of fruity or oniony odours.

Effect of Ethanol

Many years ago it was suggested by Omelianski (1923) that a fruit-like odour might be produced by certain proteolytic bacteria splitting off valeric acid from leucine, which in turn might combine with an alcohol to form an ester. He further observed that the addition of ethyl alcohol to a peptone solution containing a culture of aromatic bacteria greatly intensified the production of the ester-like odour.

Following this suggestion of Omelianski, ethanol was added to substrates containing each of the amino acids listed in Table V. It was found that with those amino acids which normally give rise to the fruity odours, the addition of the alcohol not only intensified the odour but caused it to persist over a much longer period. There was also a slight difference in the odour itself but it was still of the "fruity" type.

Ps. perolens is an achromogenic organism, similar in many of its characteristics to *Ps. fragi*. It differs from *Ps. fragi* in producing a distinct musty and potato-like odour. On partially hydrolyzed proteins, amino acids, and on all the culture media tested it did not produce anything resembling a fruity odour. However, when 1% ethanol was added to a medium containing valine, dextrose, and inorganic salts, which was then inoculated with *Ps. perolens,* there was a stage in which a distinct fruity odour was observed. There was no fruity odour produced by this organism in the same medium minus the ethanol.

Tests were also made with methyl, propyl, butyl, and amyl alcohol. The results were not satisfactory for two reasons: in the concentrations used some of the alcohols entirely inhibited growth; also, some of these alcohols had such pronounced odours by themselves that one could not be sure whether or not they were masking other less intense odours. This phase of the work is being continued with the hope of overcoming these difficulties.

Effect of pH on Odour Development

Many tests were made to determine the effect of hydrogen ion concentration on the development of fruity and oniony odours. In general it was found that the lowest pH permitting growth of the bacteria was the best for the production of both these odours. Where a culture produced both odours from one substrate the lowest pH developed the strongest oniony odour. Where cabbage-like odours were produced these also were strongest at the lower pH values.

Although fruity odours were most intense in the pH range 6.0 to 6.7, they were also produced up to pH values of 7.4 or 7.5. Above this, they were changed to a type of odour that was described as "candy-sweet", but not fruity. It was also observed that if a typical fruity odour had already developed in a medium, the addition of a small amount of alkali changed it to this same candy-sweet type.

Oniony Odours and Sulphur Compounds

Up to the present time, the nature of the compounds responsible for these so-called oniony odours remains unknown. Nor is there any evidence suggesting the mechanism by which they are formed.

It is well established that the compounds responsible for the characteristic odours of onions and garlic belong to a group of sulphur-containing compounds which may be generally described as thio alcohols, alkyl sulphides and disulphides, and isothiocyanates.

For this reason it is interesting to note that taurine and the sulphur-containing amino acids cysteine, cystine, and methionine were not among those that produced strong oniony odours in these tests. Other tests were made in which a wide variety of organic and inorganic sulphur compounds were added to a supposedly sulphur-free substrate, with asparagine as the only nitrogen source. In the supposedly sulphur-free control medium these organisms produced traces of oniony odours when inoculated with the appropriate organisms. These odours were produced much earlier and in much greater intensity when aluminium ammonium sulphate was added. On the other hand, free sulphur, sodium sulphite, sulphate, thiosulphate and sodium sulphydrate made no difference to the odour produced. Other sulphur compounds including cysteine, cystine, methionine, taurine, glutathione, thiourea, alkylthiourea, mercaptosuccinic acid, and sodium alkyl amyl sulphate caused the production of various offensive odours, but did not produce or increase the oniony odours.

This does not mean that these particular oniony odours are not sulphur-containing compounds. Minute traces of sulphur might have been present in impurities in the ingredients used or the inoculum itself might have contained enough sulphur to produce these odours. It does suggest that some specific type of reaction rather than the mere presence of sulphur in the substrate is the important thing.

These same sulphur-containing media were also inoculated with the fruity cultures 4 and B1. Very strong fruity odours were produced in the one containing aluminium ammonium sulphate and quite strong fruity odours in those containing thiourea and alkyl thiourea.

DISCUSSION

Those interested in the problems of fish muscle spoilage have always given particular attention to the non-protein nitrogen extractives. Early workers demonstrated the importance of trimethylamine oxide in the fresh muscle and the accumulation of tri- and di-methylamine and other volatile bases as muscle deteriorates. At that period very little was known about the presence of free amino acids in fresh muscle and it was taken for granted that spoilage involving decomposition

of amino acids must necessarily be preceded by the breakdown of the fish muscle proteins. For this reason, these earlier workers believed that spoilage of fish muscle occurs in two stages — first, the reduction of trimethylamine oxide coupled with the oxidation of lactic acid; and second, the hydrolysis and subsequent decomposition of the proteins.

In spoilage of most sea fish these two stages of decomposition do take place, but they are only part of a more complex series of changes. With increasing knowledge of the chemical components of fresh muscle and with a better understanding of the chemical activities of fish-spoiling bacteria, this over-simplified picture of spoilage must undergo some modification. It would appear that some of these modifications may be related to the action of bacteria on other nitrogenous materials normally present in the fresh muscle juice.

During recent years increasing attention has been given to the presence of free amino acids and closely related compounds in fish muscle before it has undergone and deterioration (Shewan, 1951). Kutscher and Ackermann (1936) demonstrated that glycine and alanine were invariably present in extracts made from the muscle of many marine species. Fougère (1952) found twelve amino acids in the free state in cod muscle juice. Shewan (1953) and Shewan *et al.* (1952, 1953) have not only demonstrated the presence of many different free amino acids in fish muscle, but have also shown that there is a difference in the distribution of these amino acids in two of our major classes of fish, the Teleostei and the Elasmobranchii.

With these thoughts in mind it is of interest to note that *Ps. fragi* neither reduces trimethylamine oxide nor hydrolyzes proteins, but it does contribute significantly to the early spoilage odours in fish. The results recorded in this paper indicate that the sweet, fruity, oniony, and vegetable-like odours produced by *Ps. fragi* are the result of its action on different amino acids. It is conceivable that most, if not all, of the early spoilage odours listed by Shewan *et al.* (1953) are produced by organisms similar to *Ps. fragi* acting on these nitrogenous extractives.

In his book "The Microbiology of Meats", Jensen (1954) speaks of testing bacteria for their ability to produce esters by growing them in a nutrient broth to which dextrose and various alcohols were added. In this way organisms isolated from meats produced odours suggesting amyl valerate (apple), ethyl butyrate (pineapple), amyl acetate (banana), and ethyl acetate (fruity).

The question arises as to how organisms such as *Ps. fragi* can act on amino acids to produce both fatty acids and alcohols. The former can be produced by a number of different reactions involving deamination, while decarboxylation could result in the production of a primary alcohol. But, as pointed out by Gale (1940) the optimum pH values for deamination and decarboxylation are such that one would not expect them to occur simultaneously. It is possible, however, that decarboxylation takes place first, followed by deamination as the pH increases. This might be an explanation of why an initial slight acid reaction favours the production of strong fruity odours, while at initial pH values above 7.5 they do not develop.

The observations made with *Ps. fragi* and *Ps. perolens* suggest that if these fruity odours are derived from esters, the latter organism differs from the former in its inability to produce the necessary alcohol. *Ps. fragi* produced typical fruity odours with an amino acid as the only precursor; *Ps. perolens* produced a similar ester-like odour only when an alcohol had been added.

This discussion does not imply that in spoiling fish muscle the amino acids are necessarily the sole source of either the alcohol or the acid that might combine to form esters. Either could be produced from a number of other sources, or they might be produced separately by two entirely different organisms.

It is also important to note that even with relatively simple substrates, such as single amino acids or mixtures of them, the odours produced by *Ps. fragi* are not confined to the sweet and fruity types. On more complex media, such as peptones or protein hydrolysates, it invariably produces a succession of different odours. Occasionally attempts to produce fruity odours with *Ps. fragi* on fish muscle have been unsuccessful. Apparently the necessary free amino acids were not present in sufficient quantity or some other condition necessary for ester formation had not been fulfilled. One cannot help but wonder how leaching and prolonged washing or soaking will affect the free amino acid content of fish muscle, and how this in turn may affect the subsequent spoilage pattern as indicated by odour formation.

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Proteins in Fish Muscle. 12. Ultracentrifuge Studies on Post-rigor Extracts of Structural Protein[1, 2]

By D. G. Ellis and P. M. Winchester[3]

Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

Ultracentrifuge examination of extracts of post-rigor Atlantic cod muscle showed the actomyosin fraction to consist of one major component, three minor components, and a gel fraction. At ion concentrations above 0.9 the major component dissociates to form two of the minor components.

INTRODUCTION

The importance of changes in the actomyosin system of proteins during frozen storage of fish has been shown by Dyer (1951, 1953). Loss of protein extractability parallels organoleptically determined grades during prolonged frozen storage. Little is known of the physical properties of these proteins before or after the changes occurring during frozen storage.

The ultracentrifuge has proved to be a useful tool in the study of these proteins as it permits examination of the system as a whole, giving both a rapid qualitative picture of the components and, in the sedimentation constant, a physical property of each component of the system. The sedimentation constant is related to the size and to the shape of the protein molecule. To understand the changes of muscle protein during frozen storage, as followed by ultracentrifuge technique, the properties of these proteins before freezing must be known. Since most commercially frozen fish have passed through rigor prior to freezing, in this present study post-rigor fish muscle was examined before pre-rigor muscle.

The chemistry of the structural protein of muscle has been reviewed in detail by Bailey (1954). Most of the work covered in that review is concerned with mammalian muscle. Hamoir (1955) has reviewed the field of proteins of fish. Fish muscle contains a globulin fraction consisting of actomyosin, myosin, actin, and tropomyosin or nucleotropomyosin, also an albumin fraction of many components, as in the case of mammalian muscle.

Dyer *et al.* (1950), using cod muscle, found the protein content to be 18%, of which about 70% belonged to the actomyosin system.

Johnson and Landolt (1950, 1951), working with rabbit muscle, found in their actomyosin preparations three components, actomyosin, myosin, and actin. They have postulated the existence of a reversible dissociation:

$$\text{Actomyosin} \rightleftarrows \text{Actin} + \text{Myosin}$$

influenced by salt concentration and adenosine triphosphate.

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[1]Received for publication June 10, 1958.

[2]Part 11 of this series appeared in this Journal, **15**(2): 135–153, 1958.

[3]Present address: Department of Fisheries, Fish Inspection Laboratory, Halifax, N.S.

EXPERIMENTAL

MATERIAL

Striated skeletal muscle from cod fillets (*Gadus callarias*) was used for preparation of protein solutions. The gutted fish were obtained from the shore fishery and were generally 6 to 12 hours in ice. They were stored at 0°C until used. All fillets used had passed through rigor before protein extraction was carried out.

PREPARATION OF SAMPLES

Protein extraction was carried out using the method of Dyer *et al.* (1950), with minor modifications. Thus the extraction solution was sodium chloride adjusted to the desired pH, normally 7.0, with NaH_2PO_4–Na_2HPO_4 mixtures. The ion concentration ($\Gamma/2$) of the buffered solution was 0.6, and the total phosphate concentration in all cases was 0.05 M.

(The ion concentration ($\Gamma/2$) is defined as

$$\frac{\Sigma_i C_i Z_i^2}{2}$$

where C_i is the concentration of ion species i in moles/litre and Z_i is the charge of ion species i. Both positive and negative ions are considered. In dilute solution $\Gamma/2$ is numerically the same as the ionic strength (μ), which is defined by

$$\frac{\Sigma_i M_i Z_i^2}{2}$$

where M_i is the concentration of ion species i in moles/kilogram of solvent.)

Potassium chloride extraction solutions, $\Gamma/2 = 0.6$, were also used. Results did not differ from those obtained with sodium chloride.

The extraction solution was chilled until ice was present, before blending. Blending was carried out with a Waring blendor at reduced speed, about 7,000 rpm, to reduce cavitation effects. Total blending time was 90 sec, in nine 10-sec periods separated by 5-sec periods, to allow unextracted tissue to settle. The extract was centrifuged at 0°C for 10 min at a relative centrifugal force of $1000 \times g$. The supernate was the "Total Soluble Protein" extract used later in this work.

The actomyosin fraction was precipitated from the cold Total Soluble Protein extract by diluting with nine volumes of distilled water at room temperature and centrifuging immediately for 10 min at a relative centrifugal force of $1000 \times g$. The precipitate was re-dispersed immediately in buffered salt solution.

The protein content of the Total Soluble Protein and actomyosin solutions was determined by the biuret procedure calibrated against the microkjeldahl method (Snow, 1950).

Chloride, in the presence of protein, was titrated with silver nitrate, using dichlorofluorescein indicator (Dyer, 1943).

A Spinco Model E analytical ultracentrifuge was used for velocity sedimentation measurement on the protein solutions. Several runs made in the temperature range of 5 to 10°C showed no differences from runs made at 20°C. All further runs were therefore made in the 20°C range (18 to 21°C).

No corrections of the sedimentation constants for viscosity were made. Such corrections are usually of major significance. However, the protein solutions used were known to consist of a mixture of three or four components which have not as yet been separated and identified. The viscosity behaviours of these components are believed to be quite different; so for the present an uncorrected, or apparent, sedimentation constant s' is used. This sedimentation constant is given in Svedberg units, S (cm/sec/dyne/$g \times 10^{13}$).

Protein solutions could be stored, generally, a week at 0°C without change in sedimentation constant, or in appearance of the ultracentrifuge pattern. When change did occur, it appeared to be the result of aggregation and precipitation of protein. The resulting sedimentation constants and the ultracentifuge patterns corresponded to those expected from the reduced protein concentration.

RESULTS

The Total Soluble Protein extract from which the actomyosin solution was prepared also contained the albumin group of proteins. These proteins sediment slowly and the peaks diffuse rapidly in the ultracentrifuge. During ultracentrifuge runs of short duration, the albumins did not affect either the pattern shown by the actomyosin group (Fig. 1–b) or their sedimentation rate, when concentration was considered. Consequently, the Total Soluble Protein extract was used in much of this work, eliminating the precipitation of actomyosin. This precipitation was the

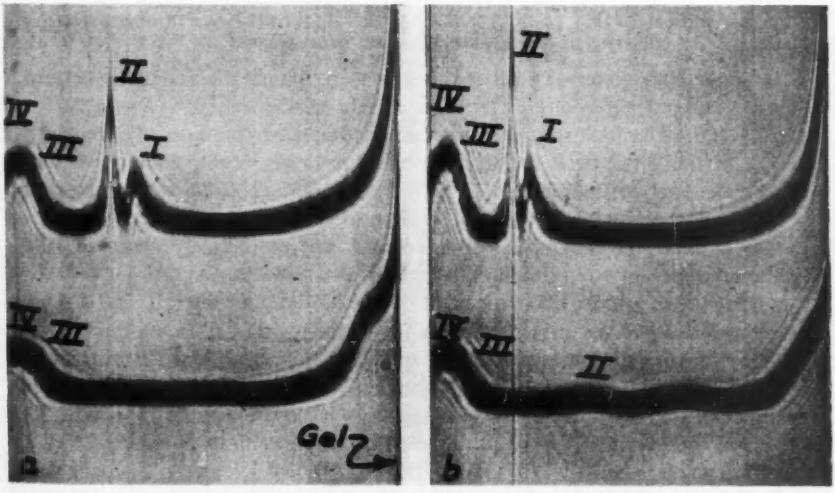

FIG. 1. Sedimentation diagram of (a) actomyosin and (b) Total Soluble Protein, in salt solution, $\Gamma/2 = 0.6$, pH = 7.0.
Sedimentation to right.
Centrifuge speed = 59,780 rpm. Bar angle 65°.
Time 12 min. Temperature 20°C.
Upper curve, protein concentration 0.98 mg N/ml.
Lower curve, protein concentration 0.49 mg N/ml.

weakest link in the preparative procedure, frequently resulting in a broadening of the peaks of the ultracentrifuge pattern, probably due to increasing polydispersity. The longer the protein was left in the precipitated form the more difficult it became to re-disperse, and the polydispersity increased.

The actomyosin fraction prepared from the Total Soluble Protein extract when examined in the ultracentrifuge was found to contain a gel fraction and at least three other components. A typical ultracentrifuge photograph is shown in Fig. 1–a.

Component II was found to be the major constituent. Its very sharp peak showing very slow spreading, its high sedimentation rate, and the large concentration dependence of the sedimentation constant, indicated a very asymmetric molecule of large size. The sedimentation constant, extrapolated to zero protein concentration, was found to be 60 S.

Components III and IV were present in small amounts. They sedimented slowly, showed rapid diffusion or spreading of the peaks, and little concentration dependence of the sedimentation constant, indicating a smaller size and a more symmetrical shape than component II. The sedimentation constant of component IV was found to be 2.5 S. The shape of the sedimentation constant–concentration curve for component III at low concentrations made extrapolation to zero concentration difficult. At moderate concentrations the value was 6.0 S, but the extrapolation could give a value of 9 S or higher.

A gel fraction was also present. It was removed from solution by the time the ultracentrifuge reached operating speed (60,000 rpm). Preparative ultracentrifugation indicated this gel fraction constituted about 20% of the actomyosin fraction.

Another component (I) was present in some preparations. When present it was observed only in solutions of high protein concentration — above 0.75 mg protein N/ml. The high viscosity of these concentrated solutions may reduce the diffusion of the small peak enough to permit observation. Or component I may dissociate at low concentrations. The sedimentation constant was greater than that of component II, but it was not possible to extrapolate to zero concentration with the limited data available. Component I was more easily observed in the Total Soluble Protein extract than in the actomyosin solution.

Attempts to prepare component II free from components III and IV were not successful. Repeated precipitation of the actomyosin fraction, by dilution, and re-solution in salt solution, resulted only in loss of sharpness of peaks of the ultracentrifuge pattern. Component II could be removed from the solution by preparative ultracentrifugation, leaving only components III and IV in the supernate. The precipitated component II could, with difficulty, be partially re-dispersed. A dilute solution of components II, III, and IV resulted. This indicated a dissociation of component II into components III and IV, although there was the possibility of some III and IV being carried down with component II.

Attempts to concentrate the dilute solutions of components III and IV by ultrafiltration resulted in the aggregation of component III to several larger species. Component IV was not affected.

Protein Concentration

The effect of protein concentration on the apparent sedimentation constants of the four components is shown in Fig. 2 and 3. The concentration values used in the Figures are those of the total protein content of the solutions, not those of the individual components. Since the component boundaries in the ultracentrifuge cell corresponding to the four components sediment outward in order from I to IV, the protein solution through which each component boundary sediments will be

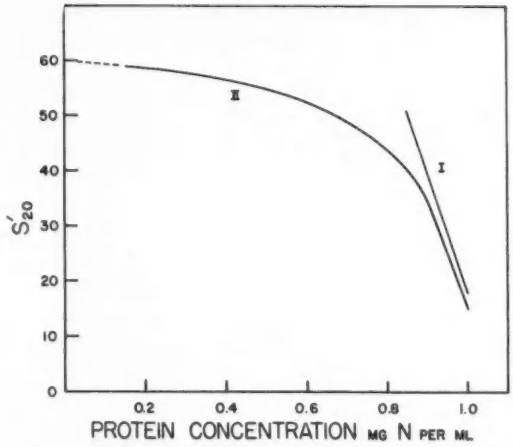

Fig. 2. Sedimentation constants *vs.* total protein concentration for components I and II. Actomyosin in salt solution $\Gamma/2 = 0.6$, pH = 7.0. This curve is typical of the results obtained in many runs. Considerable scatter of points is usually obtained as expected for this particular procedure.

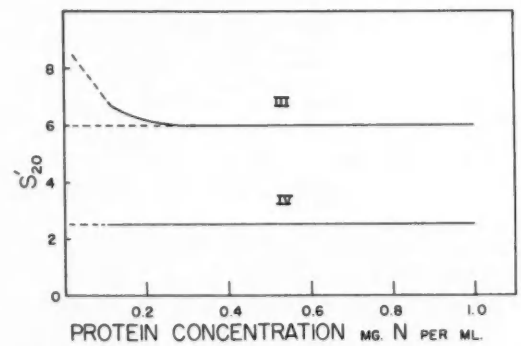

Fig. 3. Sedimentation constants *vs.* total protein concentration for components III and IV. Actomyosin in salt solution, $\Gamma/2 = 0.6$, pH = 7.0. This curve is typical of the results obtained in many runs. Considerable scatter of points is usually obtained as expected for this particular procedure.

different in the number of components it contains and in the total protein concentration. The sedimentation constant of each component will thus be measured in a solution of different total protein concentration and viscosity, with the resultant variation in value. Thus the relative values of the sedimentation constant for the various components cannot be compared on the basis of total protein concentration. This is a problem associated with the use of the ultracentrifuge with any unknown mixture of components. The s' values extrapolated to zero concentration, however, may be directly compared. This extrapolation also largely eliminates the error introduced by not correcting the observed sedimentation constants for viscosity effects.

Effect of pH

Within the range 6 to 8, the effect of pH is small, both on the sedimentation constants and on the appearance of the ultracentrifuge pattern. For component

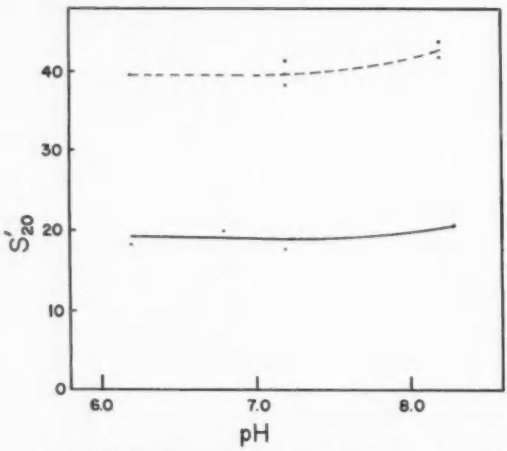

FIG. 4. Sedimentation constants *vs.* pH for component II.
Total Soluble Protein extract in salt solution, $\Gamma/2 = 0.6$.
- - - Protein concentration = 0.37 mg N/ml.
—— Protein concentration = 0.70 mg N/ml.

II, there is a slightly higher sedimentation rate at pH 8 than at pH 6 or 7. Typical values are shown in Fig. 4. Component I appears to behave similarly in this respect.

There was no change of sedimentation rate for components III and IV over this pH range. Component III became less stable, however, at pH 8, showing a tendency to form aggregates with sedimentation constants in the 9 to 12 S range.

Ion Concentration

Component II was stable in solution in the ion concentration range 0.4 to 0.9. Above an ion concentration of 0.9 to 1.0 a dissociation of component II to give component III and IV (Fig. 5), became apparent. This dissociation was practically

complete at $\Gamma/2 = 1.6$ to 2.0. In dissolving precipitated protein of the actomyosin group, with its high water content, solvents of high ion concentration ($\Gamma/2 = 1.5$) were used to reach a final ion concentration of 0.6. Under these conditions the protein was exposed to ion concentrations above the critical level for the few minutes before mixing was complete. No apparent dissociation was found. After the dissociation occurred it was not reversed by lowering the ion concentration.

In extracts containing component I, exposure to high ion concentration resulted in its disappearance as well.

Below $\Gamma/2 = 0.4$ the solubility of the actomyosin system of proteins was reduced until at $\Gamma/2 = 0.24$ the solubility was negligible (see also Dingle, 1958).

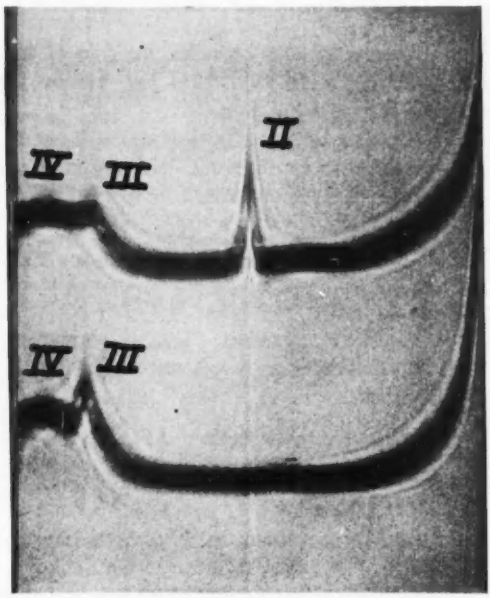

FIG. 5. Sedimentation diagram of actomyosin in salt solution, $\Gamma/2 = 1.2$, pH = 7.0.
Sedimentation to right.
Centrifuge speed = 59,780 rpm. Bar angle 65°.
Time 32 min. Temperature 20°C.
Upper curve, protein concentration 0.95 mg N/ml.
Lower curve, protein concentration 0.88 mg N/ml.

Sedimentation behaviour in this region of ion concentration was erratic. Under some conditions component II aggregated, reaching sedimentation constants up to 200 S. This aggregation may occur during preparation or handling of the protein if the ion concentration is allowed to fall below 0.4. While the aggregation was not strictly irreversible, re-dispersion was difficult.

DISCUSSION

Extracts of the structural protein of fish muscle, the actomyosin system, have been found to contain four components and a gel fraction. The gel has not yielded

further constituents. It is present in a consistent quantity, which may suggest it to be a distinct fraction or component rather than denatured protein produced during extraction and handling procedures.

The other components are stable in solution when held at 0°C and at an ion concentration of 0.6 to 0.8. Raising the ion concentration above 0.9 — not a particularly harsh treatment — dissociates component II, giving components III and IV, and there is some indication that gel is produced as well. Originally an extraction solution having an ion concentration of 0.8 was used in this work. Excessive freezing of the solution, as used in the extraction procedure, could raise the ion concentration above the critical range ($\Gamma/2 = 0.9$ to 1.0) resulting in partial dissociation of component II. The use of an ion concentration of 0.6 provided an adequate safety factor.

Component II thus seems to be a complex, built up of units of components III, IV, and possibly of the gel fraction, held together by forces moderate in character, yet stable under normal conditions.

Since an appreciable dissociation of component II occurs at elevated ion concentrations, it is reasonable to expect some dissociation at normal ion concentrations ($\Gamma/2 = 0.6$ to 0.8). This is supported by the fact that component II has not been prepared free from III and IV, nor has it been separated from them. We then have a dissociation

$$\text{II} \longrightarrow \text{III} + \text{IV} (+ \text{gel})$$

which is influenced by ion concentration. This is similar to the equilibrium proposed by Johnson and Landolt for rabbit muscle protein in the presence of salt or adenosine triphosphate:

$$\text{Actomyosin} \rightleftarrows \text{Actin} + \text{Myosin}.$$

The dissociation of component II, however, has not been found to be reversible under our conditions.

The components found to be present in fish muscle have not been identified in terms of the constituents of mammalian muscle. On the basis of sedimentation constants only, we might associate component II with actomyosin, component III with myosin, and component IV with either tropomyosin or globular actin — all of which have been found in fish muscle protein (Hamoir, 1955). The ion concentration present in our solutions (0.6) is high for the existence of globular actin in active form. The fibrous form of actin could be identified with the gel fraction present in the actomyosin solution. This offers an interesting possibility, fitting both actin and tropomyosin into the actomyosin complex and the subsequent dissociation mechanism.

SUMMARY

The actomyosin system of proteins of post-rigor cod muscle consists of one major component, three minor components, and a gel fraction. Since the major component and one minor component have high sedimentation constants, very sharp slowly spreading peaks in the ultracentrifuge pattern, and large concentration

dependence of the sedimentation constant (15 to 60 S for component II), they are believed to be very large and very asymmetrical molecules.

At moderate ion concentrations (0.4 to 0.9) these proteins are stable in solution at 0°C for a week or more, and for a short time at room temperature.

Above an ion concentration of 0.9 the large protein component dissociates irreversibly to yield the smaller ones.

The two small protein components have always been found to be present with the large protein components. Their presence may be explained by a dissociation of the large molecules.

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Proteins in Fish Muscle. 13. Lipid Hydrolysis[1, 2, 3]

By W. J. Dyer and Doris I. Fraser

Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

Lipid hydrolysis with formation of free fatty acids occurs rapidly in frozen cod fillets at +10°F, and slowly at −10°F. In fatty fish the free fatty acid development is slower but still temperature dependent. It appears that when appreciable lipid hydrolysis occurs on storage usually the actomyosin extractability, as well as taste panel scores, decreases. Contrary to most previous results, there was no loss in actomyosin extractability or taste panel acceptability up to a year and a half in the frozen cod at −10°F.

Recent results obtained in this laboratory have indicated that the lipids of some species of fish are hydrolyzed in frozen storage (Dyer and Morton, 1956; Dyer *et al.*, 1956). The degree of hydrolysis appeared to be related to the stability of the protein and its extractability in salt solution. The results also showed that fat spoilage was involved in the deterioration occurring under frozen storage, even in the non-fatty species (Dyer, 1951, 1953).

While considerable research has been carried out on fat oxidation, very little appears to have been done on free fatty acid (FFA) formation. Some early work on various species of Pacific salmon (Brocklesby, 1933) showed a gradual increase in FFA on frozen storage, from acid values of 0.1 or less on the fresh and freshly frozen fish to values of about 5 at 3 months, 8 to 14 at 6 months to a year, with a few values of 40 to 80 at 2 years storage. (These acid values × 0.5 equal percentage FFA of the extracted fat.)

This communication reports the findings on the formation of FFA in several Atlantic species: cod (*Gadus callarias*), plaice (*Hippoglossoides platessoides*), halibut (*Hippoglossus hippoglossus*), and rosefish (*Sebastes marinus*), with special emphasis on results obtained on frozen cod stored at +10°F (−12°C) and at −10°F (−23°C).

Lipids Present in Fish

Lovern and co-workers, in a recent series of publications (see Garcia *et al.*, 1956), have shown that the flesh lipids of cod and haddock contain very little triglyceride fat, approximately 3% of the total lipid. The remainder consists of about 35% choline phosphatides, about 7% ethanolamine phosphatides, no serine phosphatide, 13% cholesterol and cholesterol esters, 13% waxes and alcohols, 6% FFA, 2% inositol lipids, and about 21% unidentified lipid. The total lipid as

[1]Received for publication, July 8, 1958.

[2]Part 12 of this series appears in this issue, pp. 33–41.

[3]A part of this work was presented in summary form at the Atlantic Fisheries Technological Conference, Boston, Mass., January 28, 1958.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

extracted by a series of solvents was about 0.6% in both cod and haddock. This is of the same order as that found for cod by Dambergs (1956) using the ether-soluble fraction of an acetone–water extract.

RESULTS

Formation of Free Fatty Acids in Stored Frozen Plaice, Halibut, and Rosefish

Development of FFA in stored frozen plaice, halibut, and rosefish is shown in Fig. 1. The results are expressed as percentage FFA of the extracted fat (as oleic acid). The samples were extracted with chloroform in the presence of sodium sulphate and the extracts titrated with alkali as previously described (Dyer and

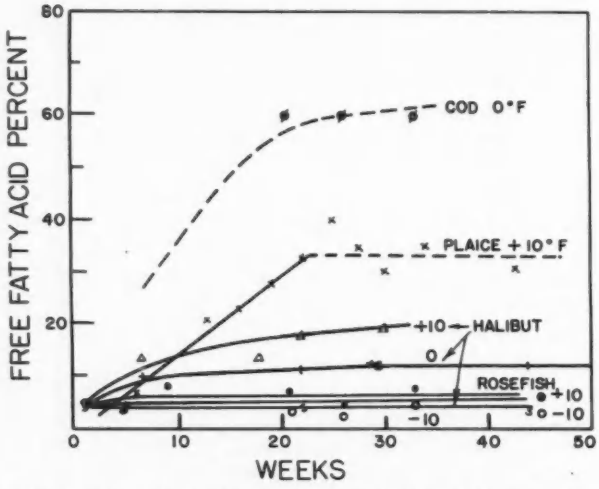

Fig. 1. Free fatty acid development in stored frozen plaice, halibut, rosefish, and cod.

Morton, 1956). In plaice stored at +10°F, the development was quite rapid, reaching levels of about 35% at 25 to 30 weeks. In halibut at +10°F, development was slower, reaching about 20% at 30 weeks, whereas at 0°F (–18°C) it remained at about 10% from 2 to 8 months; at –10°F, it held at a level of about 2% throughout the storage period. Rosefish behaved similarly, FFA remaining at about 4% at +10°F and 2% at –10°F. Insufficient samples were analyzed in the early phases of the storage period to establish the initial level of FFA.

The results show a remarkably good relation with deterioration in quality as measured by taste panels and with the protein extractability curves previously reported (Dyer, 1951; Dyer and Morton, 1956; Dyer *et al.*, 1956). The production of FFA appears to be related to the protein denaturation.

Scattered results on frozen cod, which exhibited a rather rapid loss of protein solubility, showed even more rapid formation of FFA. Consequently, experiments were set up to determine the rate of FFA formation in unfrozen iced fish and in stored frozen cod fillets.

FFA Formation in Gutted Cod Stored in Ice

One lot of gutted market cod (shore fish), iced within 2 hours after catching, was stored in ice at the laboratory. Samples were taken, usually daily, filleted, and the muscle was analyzed for trimethylamine (Dyer, 1945, 1950) and for FFA in

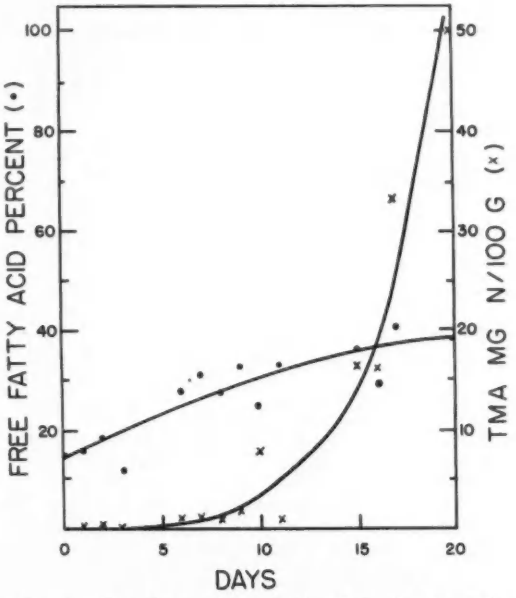

Fig. 2. Free fatty acid and trimethylamine formation in gutted cod stored in ice.

the fat, as described above. The initial FFA value of 15% gradually increased to a level of about 30% at 10 days and 35% at 15 days (Fig. 2). This slow increase of FFA apparently would not be satisfactory as a spoilage test. The trimethylamine curve was similar to that usually obtained (Dyer *et al.*, 1946).

Presumably the hydrolysis of the lipid is due to lipase enzymes, but whether these are present in the muscle or are produced by the bacteria present is not yet known (cf. Lea, 1957). Preliminary results indicate that the acids formed are non-volatile and thus the hydrolysis is probably due to a lipase acting on the higher fatty acid esters of the lipid fraction rather than to an esterase acting on esters of the lower fatty acids (Nachlas and Blackburn, 1958).

Several other determinations on fresh cod fillets showed initial values of from 10 to 15% FFA in the fat. Cardin and Bordeleau (1957) also report values in this range.

FFA Formation in Stored Frozen Cod

Two lots of gutted cod were obtained, the first lot having been 1 day, and the second lot 8 days, in ice on a trawler. The fish were filleted, packed in 1-lb cartons, and quick-frozen in a local fish plant. One 24-carton box of each lot was placed at +10°F and another at –10°F. The storage temperatures were reasonably constant,

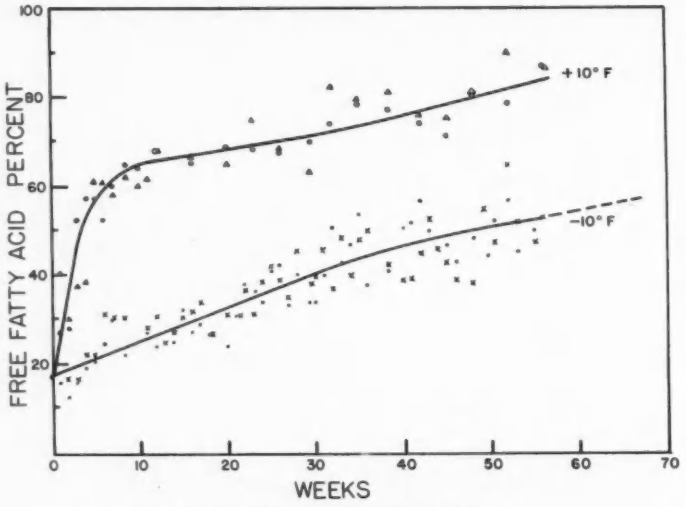

FIG. 3. Free fatty acid in stored frozen cod:
○ 1 day in ice prior to freezing, stored at +10°F;
● 1 day in ice prior to freezing, stored at –10°F;
△ 8 days in ice prior to freezing, stored at +10°F;
× 8 days in ice prior to freezing, stored at –10°F.

varying over a range of about half a degree (F) or less throughout the duration of the experiment. Periodic analyses of moisture, extractable protein, actomyosin, FFA, and peroxide, were carried out as described previously (Dyer *et al.*, 1956).

The moisture content of the 1-day-iced fish averaged 81.5(±0.74)% (23 samples), and the 8-day-iced fish, 81.3(±0.45)% (25 samples), in agreement with previous results (Dyer *et al.*, 1957a, b). There was no drying out during the course of the experiment at either temperature. The trimethylamine nitrogen content of the 1-day fish was 0.5 mg per 100 g, that of the 8-day fish, 6.0.

The initial FFA value was about 15%, in agreement with the results on fresh fish. The data in Fig. 3 indicate that the rate of formation of FFA was dependent on temperature rather than on the time the fish was stored in ice prior to freezing. At a given temperature, the rate was the same for the 1-day and the 8-day-iced

fish. At +10°F, development of FFA was very rapid, reaching about 55% (expressed as oleic acid in the extracted lipid), after storage for 4 weeks. The rate then slowed down, the level approaching 90% after 60 weeks. At –10°F, the FFA increased much more slowly, almost linearly to a level of about 55% at the termination of the experiment after 55 weeks.

The amount of lipid extracted varied non-systematically from 0.1 to 0.25 g per 100 g muscle (i.e. about one quarter of the total lipid present), regardless of storage temperature.

In the later stages of the experiment, some of the samples were extracted by a recently developed procedure using chloroform–methanol as solvent (Bligh and Dyer, unpublished). This gave a yield of about 0.5% lipid. The FFA values in

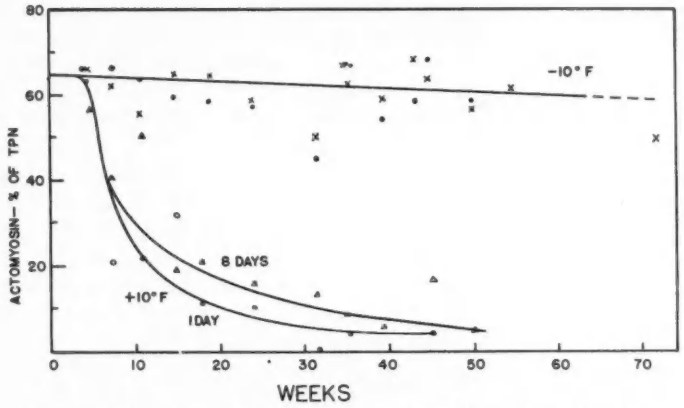

FIG. 4. Actomyosin extractability, percentage of total protein nitrogen, in stored frozen cod. (Legend as in Fig. 3.)

such extracts were about 65% between 62 and 72 weeks in the samples stored at + 10°F, and about 28% in those stored at –10°F for the same period.

The extractable actomyosin (Fig. 4) was expressed as percentage of the total protein nitrogen of cod muscle which was taken to be 2.5% of the wet weight of tissue (Dyer *et al.*, 1957a, b). At +10°F, there was no change up to 4 weeks, but this was followed by a very rapid drop from 65% to 30% extractable actomyosin in the next 4 weeks. The curves then gradually sloped down to a level of about 4%, the 1-day-iced fish decreasing at a somewhat faster rate than the 8-day fish.

At –10°F, however, in contrast to most previous results, there was no decrease up to 70 weeks, when the samples were all used. No difference was evident between the 1-day and 8-day fish. In addition, taste panels conducted on these samples showed scores equivalent to the fresh fish!

The peroxide value of the extracted lipid was also determined (Dyer *et al.*, 1956). There was considerable scatter in the values, as is often the case, and this was accentuated by the low concentration of lipid in the extract. The samples from the fish stored at –10°F were analyzed weekly, and the means of all the

values in successive 5-week periods are plotted in Fig. 5. There were fewer samples from the +10°F fish (analyzed each 3-week period), and here the means are plotted as moving averages for 10-week periods (5 weeks on either side) at 5-week intervals. The standard error of each mean is indicated above and below each point plotted.

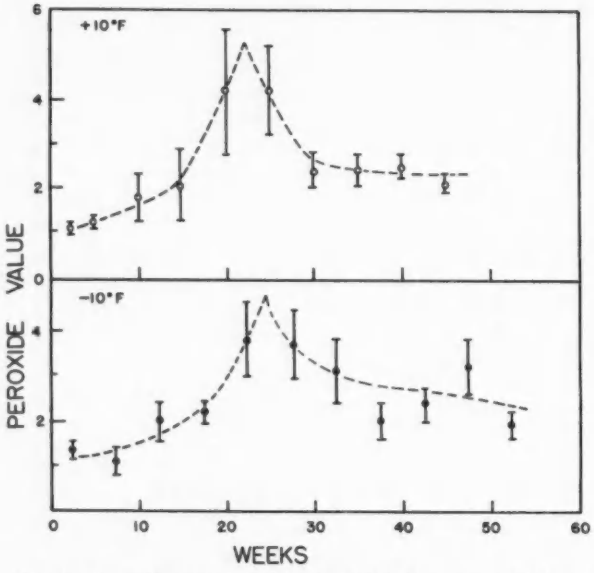

FIG. 5. Peroxide values of the extracted lipid, m-eq per 100 g, at +10°F and at −10°F, as means of results for 5-week periods (see text, above). Limit lines show standard error of the means.

While there appears to be an increase in peroxide value, with a maximum at about 6 months, followed by a decline in both the +10°F and −10°F samples, this is probably not statistically significant. Thus, we can only conclude that there is some increase in peroxide value, with a possible maximum at about 6 months.

DISCUSSION

LIPID HYDROLYSIS

The rate and extent of hydrolysis of the lipid were appreciably greater in frozen cod than in the other species so far examined. At +10°F the FFA reached a value of 65% in the first month. Information is lacking on the composition of the initial 15% FFA, and on the source and composition of the increase.

The possibility of selective extraction of the FFA or the lipid by a particular solvent should be considered, since only about one quarter of the lipid present was extracted by the solvent used. Increased extraction of both lipid and FFA was obtained by the use of chloroform–methanol as a solvent and there appears to

have been some selective extraction of fatty acids by the chloroform–sodium sulphate method. However, the scattered observations with the new procedure did show that the FFA values ran reasonably parallel in both procedures, and were about 20% lower with the chloroform–methanol solvent. It would appear that the extent of hydrolysis of the lipid present is at least 50 to 60% (at +10°F). This compares with values of less than 10 to 20% in halibut, and almost nil in rosefish.

There is as yet no information on the mechanism of the hydrolysis. The rate is much faster at +10°F than at –10°F, but the rates at +10°F and +32°F (iced fish) are almost the same. Changes resulting from the freezing-out of ice (Dyer *et al.*, 1957a) in the frozen samples apparently offset the temperature effect in the +32 to 10°F range. Presumably, the hydrolysis is an enzymic reaction (Lea, 1957) involving the phospholipids, the triglycerides, and probably some of the other lipid constituents. Whether the enzyme is present in the muscle tissue or results from bacterial contamination is not known. Since the 1-day and 8-day-iced samples, which differed very markedly in degree of bacterial spoilage, did not differ significantly in rate of FFA development, it appears probable that bacterial lipases are not involved.

Recently, Cardin and Bordeleau (1957) have reported a similar hydrolysis of the lipids during the process of salting codfish. They found approximately 50% hydrolysis of the lipids, and suggest that further hydrolysis is inhibited by high salt concentration (about 15%). In frozen fish the concentration of salts is increased greatly on freezing to the equivalent of 15% NaCl at +10°F and about 25% at –10°F (Dyer *et al.*, 1957a). These salts contain very little NaCl, but have a fair proportion of divalent salts, and the ion concentration would be higher than the equivalent concentration of pure NaCl. Thus, the lipid hydrolysis does not appear to be inhibited by salt in frozen fish.

Cardin and Bordeleau (1957) also found that little oxidation of the highly unsaturated long-chain fatty acids occurred during the salting and drying process, as determined by iodine values. This result is unexplained, since these acids should be highly susceptible to oxidation. With frozen cod, we also do not find an appreciable degree of oxidation as measured by peroxide values. There is some indication of a maximum in the curve at about 6 months, but preliminary results have not shown any indication of volatile acids or aldehydes resulting from the decomposition of the fatty acid peroxide. It appears, then, that the unsaturated fatty acids in the lipids of cod are rather stable, much more so than in many species of fatty fish, e.g. herring (Banks, 1950), mackerel, and salmon.

Protein Extractability

The very rapid drop in extractable actomyosin in cod at +10°F is in agreement with previous results (Dyer, 1951; Luijpen, 1957). The present results indicate (Fig. 4) a period of about a month at this temperature with very little change, followed by an abrupt drop and then a gradual levelling off. It is very interesting to compare this with the FFA curve (Fig. 3). At 1 month, after a rapid rise, the FFA production begins to level off just at the point where the

protein extractability curve falls. This agrees with the previous postulation of a relation between the protein denaturation and the FFA formation. In this experiment the lipid hydrolysis preceded the protein denaturation. About 50% of the total lipid is hydrolyzed prior to the denaturation. It is not yet known which lipids are hydrolyzed. If there is a relation between lipid hydrolysis and protein denaturation it may be postulated that hydrolysis destroys the stabilizing effect of the lipid on the actomyosin complex, or that the acids formed by the hydrolysis cause protein inextractability. During the storage, and accompanying denaturation, the protein loses its ability to hold moisture, and thus there is a change in the hydration characteristics of the protein micelles (cf. Seagran, 1958). This could be caused either by loss of stabilization by hydrolysis of half or more of the lipid, or by formation of a much more hydrophobic surface on the micelles of the protein by adsorption of the insoluble long free fatty acid chains.

In situ, a considerable proportion, if not all, of the lipid is probably located on the surface of the actomyosin micelles, either in a loose surface adsorption or as firmly bound lipid. When the organization of the actomyosin fibres is destroyed by peptization in salt solution, the character and the distribution of the adsorbed lipid will greatly affect the degree of peptization. Further, it is likely that when the actomyosin is peptized, a non-specific redistribution of lipid on the peptized micelles occurs, which will greatly affect its surface charge distribution, its stability in solution, and its tendency to aggregate. Thus, any hydrolysis of the lipid might be expected to greatly modify the peptization and stability of the actomyosin micelles.

During the salting of codfish, a protein denaturation similar to that in frozen fish takes place (Duerr and Dyer, 1952). When these results are compared with the lipid hydrolysis occurring during the salting process (Cardin and Bordeleau, 1957), it is apparent that formation of FFA accompanies protein denaturation as found in the present work under frozen storage conditions.

At –10°F, however, the results were quite different. Here, there was practically no decrease in protein extractability up to the end of the experiment at 72 weeks (almost a year and a half). This is most unusual for cod, since the extractability usually decreases to a low value in less than a year (Dyer, 1951). It was thus of interest to determine whether the texture and taste panel acceptability also remained constant. Several samples tested between 11 and 13 months showed no measurable difference from freshly frozen cod fillets. This also indicates that the FFA formed does not affect the taste of the cooked product, probably because these long-chain acids are quite insoluble. Two or three laboratory packs of frozen cod fillets as well as one commercial pack had been encountered previously where the protein extractability showed high values at 1 to 2 years. However, the above-described observation is the first in which this condition was found where data were available throughout the storage period.

These results are similar in part to those of Luijpen (1957) who found that the protein extractability of frozen cod fillets at –30 and –20°C remained unchanged, although some increase in toughness and decrease in taste panel acceptability occurred. Love (1956) also indicates a development of toughness

without denaturation after a year at –30°C. However, at this low temperature, both the denaturation and the deterioration as measured by taste panels would be slower (Heen, 1953).

It is significant that the denaturation did not occur with either the 1-day or the 8-day-iced fish. Thus, the freshness of the fish prior to freezing does not seem to be the responsible factor. Luijpen (1957) reports that fresh fish at –10°C. suffers considerably more protein denaturation than cod in a "condition of rather advanced spoilage". The present results at a slightly lower temperature, +10°F, do not substantiate his findings. The curve for the 8-day-iced fish is slightly above that for the 1-day fish, but the difference is probably not statistically significant.

No correlation with seasonal variations in the quality of the fish has been evident as yet. The fish used in the various experiments were found to cover almost the complete seasonal picture, with no correlation between those which suffered protein denaturation at –10°F, and those which did not.

Thus, no reason for the relatively large difference in the keeping quality of the frozen cod can so far be postulated. However, the importance to the frozen cod industry is tremendous, and efforts are being made to find the factor or conditions responsible.

SUMMARY

Development of FFA in stored frozen halibut was considerably higher at +10°F than at –10°F. In plaice at +10°F, development was more rapid, while almost none appeared in frozen rosefish. The FFA development appeared to be related to deterioration in quality on storage as shown by actomyosin denaturation and decrease in taste panel acceptability scores.

In gutted cod stored on ice, the FFA values increased to between two- and three-fold in 10 or 15 days. This is probably the result of lipase action, either originating in the muscle tissue or in contaminating bacteria.

The initial FFA value in cod was about 10 to 15%. In frozen cod stored at +10°F, this rapidly increased to values of over 50% in 1 month, and then increased much more slowly. A sharp decrease in the extractability of the actomyosin also occurred, beginning after the rapid lipid hydrolysis (about 1 month). The relation between lipid hydrolysis and protein stability is discussed.

At –10°F there was a gradual increase in FFA values to a level of 55% after a year's storage. At this temperature no decrease in protein extractability occurred, contrary to most previous data. There is as yet no satisfactory explanation for this finding, but its significance to the frozen fish industry is pointed out. There was no difference in FFA development between cod which had been iced for 1 day and for 8 days on a trawler before filleting and freezing, suggesting that enzymes present in the muscle rather than those of bacterial origin are involved.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the cooperation of W. A. MacCallum of this Station in obtaining the fish used, and in supervising the processing and freezing operations.

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Proteins in Fish Muscle. 14. Cod Tropomyosin[1, 2]

By J. R. Dingle and P. H. Odense
Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

Tropomyosin has been prepared from Atlantic cod muscle dehydrated with ethanol and ether according to Bailey's procedure. Properties measured included electrophoretic mobility, sedimentation constant, solubility as a function of pH, and viscosity. The preparations contained a small amount of nucleic acid not associated with the protein.

Tropomyosin was first described by Bailey (1946) who isolated it from rabbit muscle that had been dehydrated by organic solvents. The properties of this material were further described by Astbury *et al.* (1948), Bailey *et al.* (1948), Tsao *et al.* (1951), Bailey (1953), and Locker (1954). A similar protein has also been extracted from other muscle sources, including horse and pig cardiac muscle and whiting (Bailey, 1948), carp (Hamoir, 1951, 1952), and recently from haddock (Kubo, 1957). Among invertebrates, crystalline tropomyosin was prepared from squid (Yoshimura, 1955), oyster and *Pinna nobilis* (Bailey, 1956).

Most of the work on tropomyosin extracted from fish has been done by Hamoir (loc. cit., and 1955a, b), who prepared it in relatively small yields from frozen carp muscle by extraction with various salt solutions. By varying the ionic concentration and pH of the solutions, he extracted several proteins which he considered represented stages in the degradation of a tropomyosin complex. They contained different amounts of ribonucleic acid, and also exhibited differences in other properties such as viscosity, and in electrophoretic and sedimentation behaviour. Kubo's haddock tropomyosin (1957), prepared by Bailey's procedure, resembled rabbit tropomyosin fairly closely in electrophoretic behaviour and amino acid composition.

Tropomyosin is presumably one of the group of proteins which can be extracted from muscle with moderately strong salt solutions. Such extracts of Atlantic cod muscle, which also contain the proteins of the actomyosin system, are currently being investigated at this laboratory by ultracentrifugal methods (Ellis and Winchester, 1959). In order to assist in the isolation and identification of the components found, tropomyosin has been prepared from cod muscle dehydrated by ethanol and ether, and several of its properties have been investigated.

Materials

Fillets cut from cod (*Gadus callarias*) of various sizes were used in preparation of the protein extracts. Most of these were commercial fish that had been stored in ice for several days; in a few cases, pre-rigor fish was used, but no differences in the products obtained from pre-rigor and post-rigor muscle were apparent.

The extraction procedure was similar to that given by Bailey (1948) for assay of tropomyosin, and all steps were carried out at about 20°C. Minced muscle

[1]Received for publication August 25, 1958.
[2]Part 13 of this series appears in this issue, pp. 43–52.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

(400 g) was homogenized for 1½ minutes in a Waring blendor with 400 ml of water, and then 800 ml of 95% ethanol was added. The mixture was centrifuged at about 1300 × *g*, the residue treated with 1200 ml of 95% ethanol, and after another hour was centrifuged as before. This treatment with ethanol was repeated, and then the residue was extracted twice with 1200 ml of ether. The ether extracts were squeezed out through cheesecloth after standing for ½ hour, and the final residue was allowed to dry partially in air. While still ether-damp, 400 ml of M KCl was added and the mixture left overnight. The resulting viscous extract was squeezed out through cheesecloth, and the KCl-extraction repeated three more times, twice for 3 hours and once for ½ hour. The KCl extracts were combined, the pH was adjusted to 4.3 with N HCl, and the precipitate recovered by centrifuging immediately at 1300 × *g*. The precipitate was stirred up with 200 ml of water, neutralized with N NaOH, and the insoluble material removed by centrifuging. The yields of impure tropomyosin at this point, expressed in terms of protein nitrogen, varied between 3.7 and 4.9% of the total protein nitrogen of the fillet. The latter was taken to be 2.5% of the wet weight of the fillet (Dyer *et al.*, 1957; Dyer *et al.*, 1957).

The tropomyosin was further purified by precipitation either with ammonium sulphate (between 1.7 and 2.8 M, pH = 7), or by adding 1.5 volumes of 95% ethanol. After centrifuging at 1300 × *g* and suspension of the precipitates in water, the reagents were removed by dialysis in Visking tubing against water or weak salt solutions of pH = 7, or, alternatively in the case of the alcohol precipitate, by suspension in about 12 volumes of water, followed by reprecipitation at pH = 5.0. Electrophoresis indicated a purity of 90 to 95% for each product, and the tryptophan content was 0.043 to 0.083 g per 100 g of protein (Spies and Chambers, 1949). Such a low tryptophan content is characteristic of tropomyosin. Kubo (1957), for example, reported 0.01% for a thrice-crystallized haddock tropomyosin. On the other hand, Connell (private communication) found 0.75 and 1.57% tryptophan for cod myosin and actin respectively. Further precipitations could be carried out at pH = 4.3 without appreciable loss. The final yields of purified tropomyosin were in the range 1.8 to 2.5% of total muscle protein nitrogen.

Protein nitrogen was determined by the biuret procedure of Snow (1950); for conversion to weight of protein, a nitrogen content of 16.7% was assumed.

Electrophoresis

Electrophoresis was carried out in a Klett Tiselius-type free boundary apparatus at a temperature of 1.4°C. Samples were dialyzed at 1°C against potassium phosphate buffer solutions of ionic concentration ($\Gamma/2$) = 0.05 and required pH, containing also KCl to make up the desired total ionic concentration. In one run, Na^+ ions replaced K^+ ions.

In Fig. 1 are shown typical patterns of the product obtained after the first acid precipitation of the KCl-extract. The main peak on the ascending side was characteristically very sharp, while that on the descending side was much more diffuse. An analogous dissimilarity was found by Bailey (1948) for rabbit tropomyosin and by Kubo (1957) for haddock tropomyosin. A small rapid peak

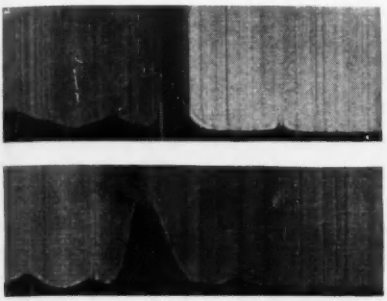

FIG. 1. Electrophoretic pattern of crude cod tropomyosin extract.

Solvent: 0.10 M KCl + potassium phosphate, total $\Gamma/2 = 0.15$, pH = 7.07. Temperature, 1.4°C. Duration of electrophoresis, 222 min. Field strength, 5.78 v/cm. Protein concentration, 1.39 mg N/ml. Top pattern, ascending; bottom pattern, descending; migration from left to right.

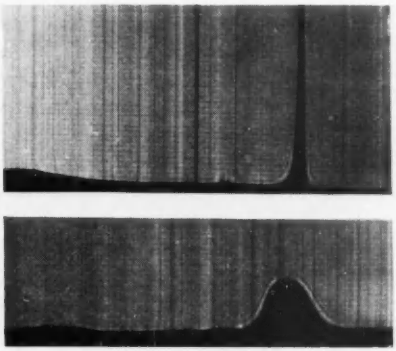

FIG. 2. Electrophoretic pattern of cod tropomyosin after precipitation with alcohol.

Solvent: 0.10 M KCl + potassium phosphate, total $\Gamma/2 = 0.15$, pH = 7.19. Temperature, 1.4°C. Duration of electrophoresis, 290 min. Field strength, 4.33 v/cm. Protein concentration, 0.826 mg N/ml. Top pattern, ascending; bottom pattern, descending; migration from left to right.

was also noticeable, as well as several slower peaks of greater size. These impurities were almost completely eliminated by an alcohol precipitation, as seen in Fig. 2. Fractionation by ammonium sulphate led to similar patterns, and reprecipitation at pH = 4.3 gave no further change. When a preparation corresponding to Fig. 1 was heated for 1 hour at 100°C and pH = 6.2, most of the slower peaks disappeared from the pattern, but the main peak became more diffuse. Similar heating of the alcohol fraction for 20 minutes caused the tropomyosin peak to become irregular in shape. This treatment was recommended by Bailey (1948) for purification in his assay procedure, but while it may cause the precipitation only of impurities, the broadening of the main electrophoretic peak suggested that the tropomyosin also suffered some change.

The variations of electrophoretic mobility of tropomyosin with pH and ionic concentration are shown in Fig. 3 and 4 respectively. This preparation had been purified by ammonium sulphate fractionation and the solvents were suitable mixtures of KCl and potassium phosphates. It was found impracticable to use a pH lower than 5.89 since even at this value some crystallization of the protein occurred in the cell. It was therefore not possible to make a direct determination of the isoelectric point by this method.

Another preparation, purified by an alcohol precipitation and a second acid precipitation, was run in a solvent containing NaCl (0.10 M) and sodium phosphates (total $\Gamma/2 = 0.15$, pH = 6.97). The mobilities ($\times 10^5$ cm^2/volt sec) were –5.72 (ascending) and –5.23 (descending), considerably lower than the corresponding values of –7.27 and –6.26 in the presence of K^+ ions (Fig. 3).

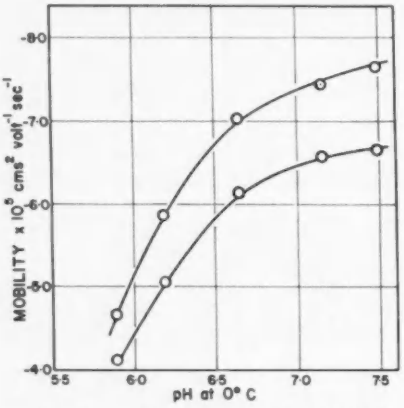

FIG. 3. Dependence of electrophoretic mobility of cod tropomyosin upon pH.
Solvent: 0.10 M KCl + potassium phosphate, total $\Gamma/2 = 0.15$. Temperature, 1.4°C. Top curve: ascending mobilities; lower curve: descending mobilities.

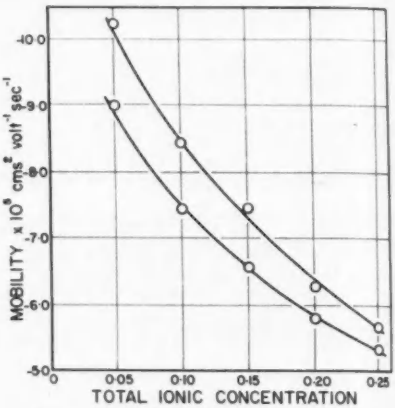

FIG. 4. Dependence of electrophoretic mobility of cod tropomyosin upon ionic concentration.
Solvent: potassium phosphate, $\Gamma/2 = 0.05$, pH = 7.10–7.27, + KCl to yield total ionic concentration indicated. Temperature, 1.4°C. Top curve: ascending mobilities; bottom curve: descending mobilities.

This difference, presumably due to a difference in adsorption of Na^+ and K^+ ions by the protein, makes it impossible to compare mobilities reported in the literature if the exact composition of the solvent is not given.

SEDIMENTATION

Sedimentation runs were made in a Spinco Model E ultracentrifuge at 59,780 rpm. The temperature of the rotor was about 20°C. The sedimentation constants (s') are given in Svedberg units for the conditions of the run, and are not corrected to water at 20°C.

A typical pattern is shown in Fig. 5 for a preparation purified by an alcohol precipitation followed by two acid precipitations. The sample appeared to be monodisperse, with a sedimentation constant of $s' = 2.1S$. A very similar pattern

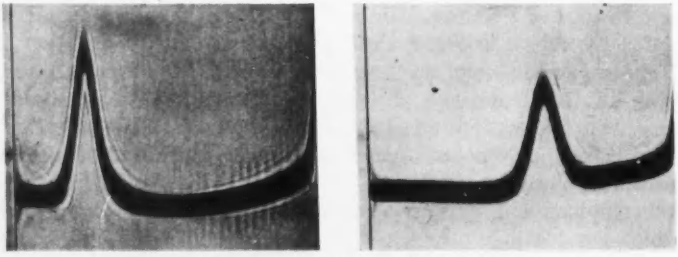

FIG. 5. Sedimentation pattern of purified cod tropomyosin.
Speed of centrifuging, 59,780 rpm. Migration from left to right. Solvent: 0.20 M KCl + potassium phosphate, total $\Gamma/2 = 0.30$, pH = 6.48. Protein concentration, 1.00 mg N/ml. Left, after 96 min.; right, after 224 min.

and constant was obtained when the K^+ ion was replaced with Na^+ ion. For rabbit tropomyosin, Bailey *et al.* (1948) found $s^\circ_{20} = 2.6S$ at a protein concentration of 0.6%, in reasonable agreement with the present value.

When the protein concentration was decreased in steps from 1.13 to 0.030 mg N/ml, the sedimentation constant increased only moderately from 1.7S to 2.3S. In this case the solvent was 0.472 M NaCl plus sodium phosphates, total $\Gamma/2 = 0.60$, pH = 7.0. (This solvent has been used at this laboratory in the ultracentrifugal study of total soluble protein extracts of fish muscle (Ellis and Winchester, 1959).) When, however, a sample was run in potassium phosphate solution, $\Gamma/2 = 0.05$, pH = 7.0, a very small peak sedimented more rapidly than the main peak, and there was a greater dependence of the constant on protein concentration. At 1.28 mg N/ml, the constants of these two peaks were 2.1S and 1.7S, and at 0.64 mg N/ml they were 3.5S and 2.4S. This behaviour may be an indication that an aggregation of tropomyosin occurs at low ionic concentrations, and would agree with the increase in viscosity noted under these conditions.

Viscosity

Measurements were made at 20°C in a modified Ostwald viscometer having a horizontal capillary of radius 0.0301 cm and length 16.0 cm. The rate of flow was varied by applying air at different controlled pressures. Over the range of average rate of shear studied (190 to 1200 sec^{-1}, depending on the protein concentration) the viscosity of tropomyosin solutions was independent of rate of shear.

The variation of viscosity with protein concentration of a preparation fractionated with ammonium sulphate is given in Fig. 6, for two solvents of $\Gamma/2 = 0.10$ and 0.30, pH = 6.50. The curves fell considerably below those reported by Tsao *et al.* (1951) for rabbit tropomyosin in the same solvent. Using the Simha equation, the intrinsic viscosities obtained from these curves, and assuming a specific volume of 0.71 cm^3/g (Bailey *et al.*, 1948), the asymmetries of the tropomyosin particles were calculated to be 33 and 27 at ionic concentrations of 0.10 and 0.30 respectively. These were probably too high, since no account was taken of their probable hydration.

Solubility

The solubility of tropomyosin as a function of pH is shown in Fig. 7. A preparation, purified by an alcohol precipitation, and two precipitations at pH = 4.3, was dialyzed so as to contain either 0.1 M or 1.0 M KCl, and no buffer. A series of mixtures of constant volume was prepared from aliquots of these stock solutions together with suitable amounts of KCl and HCl solutions to yield the desired pH and a constant KCl concentration of either 0.10 or 1.0. The ionic concentration would also be close to these values except for a small amount contributed by the HCl. The largest amount of the acid added was 5.00 ml of 0.0200 N HCl to a total volume of 25.0 ml. After standing at room temperature for 2 hours, the mixtures were centrifuged at 30,000 × *g*, and protein concentrations determined in the supernates. These concentrations were then used to calculate

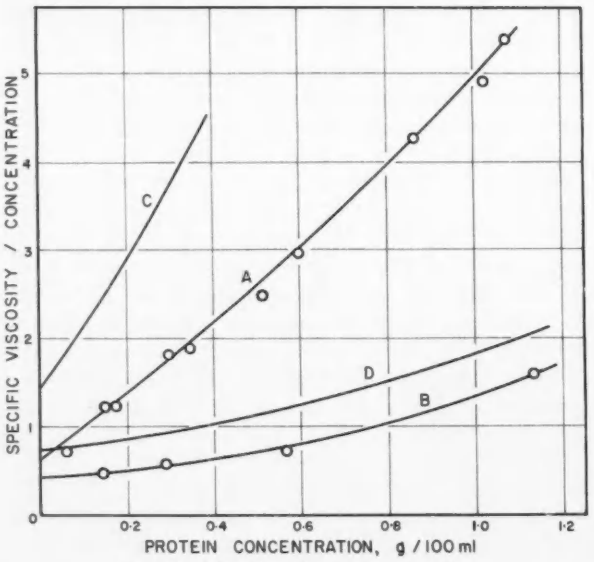

FIG. 6. Viscosity of cod tropomyosin.
Temperature, 20°C. Solvents: Curves A and C, sodium phosphate, $\Gamma/2 = 0.10$, pH = 6.50; Curves B and D, same buffer plus 0.20 M NaCl, total $\Gamma/2 = 0.30$, pH = 6.50. Curves A and B, this work; Curves C and D, taken from Tsao *et al.* (1951).

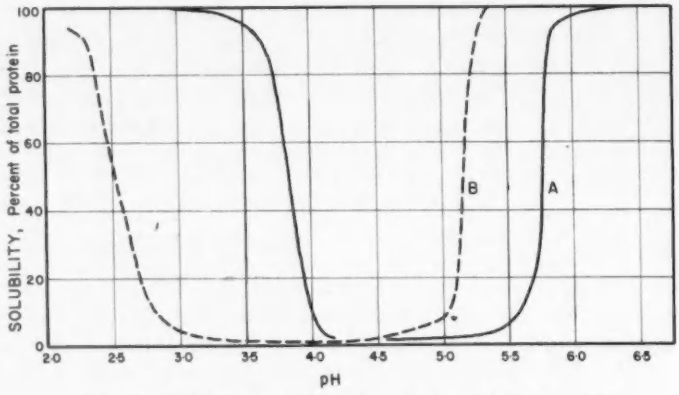

FIG. 7. Solubility of cod tropomyosin as a function of pH.
Temperature, 20°C. Solvents: Curve A, 0.10 M KCl; Curve B, 1.0 M KCl.

the percentage of the protein remaining soluble, taking the concentration at pH = 6.5 to represent 100%. (There was no precipitate at this pH.) For rabbit tropomyosin, Bailey (1948) reported that the zone of complete insolubility in 0.01 M NaCl extended from pH = 4.5 to 5.3.

Crystallization

When preparations of cod tropomyosin were dialyzed against 0.8% ammonium sulphate containing 0.01 M acetate buffer of pH = 5.4 (Bailey, 1948), crystals were obtained. In some cases they appeared as elongated hexagons, frequently in clusters, but in other cases characteristic elongated prisms were observed, as in Fig. 8. Crystals of the latter form were also obtained when a tropomyosin sample was dialyzed against NaCl–sodium phosphate, $\Gamma/2 = 0.3$, pH = 5.4 with no ammonium sulphate. These two crystal forms were once thought to represent tropomyosin and nucleotropomyosin, respectively (see, for example, fig. 3 and 2 of Hamoir, 1951). Hamoir (1955a, p. 121) has since reported, however, that

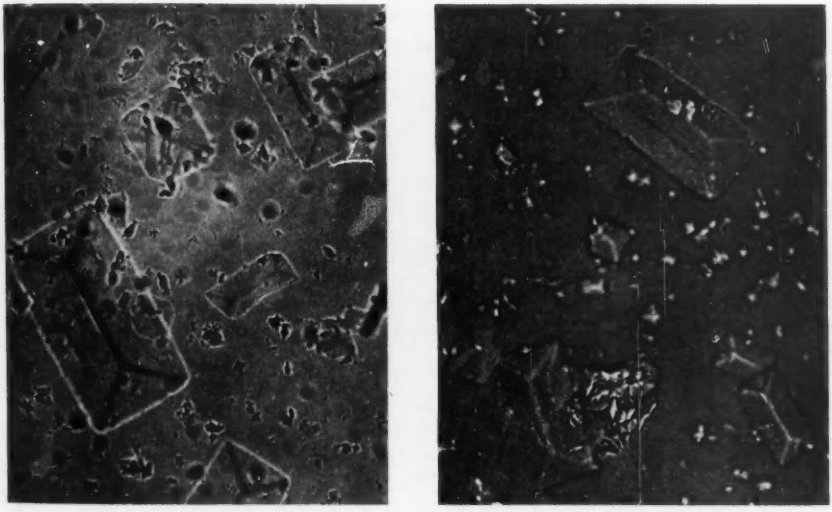

Fig. 8. Crystals of cod tropomyosin. Magnification 185×.

both he and Bailey have now observed the prism form in tropomyosins of low nucleic acid content. None of the present preparations was found to contain more than a few percent of nucleic acid. Kubo (1957) reported that it was necessary to purify haddock tropomyosin by as many as three fractionations with ammonium sulphate before prisms could be obtained. This does not agree with our experience, and the origin of the two crystal forms still remains obscure.

Nucleic Acid Content

The ultraviolet absorption curve for a sample of tropomyosin purified by an alcohol and an acid precipitation is shown in Fig. 9 (Curve A). The corresponding absorption after precipitation of the protein with 1 M perchloric acid is given by Curve B. It resembled the absorption reported by Hamoir (1955a, p. 126)

for ribonucleic acid of carp nucleotropomyosin. According to that author, the average optical density at $\lambda = 261$ mμ of such a solution containing 1 gram-atom of phosphorus per litre was 10,590. Using this value, and assuming a phosphorus content of 8.4% as reported for yeast ribonucleic acid (Smith and Markham, 1950), the nucleic acid content of our preparation was calculated to be 0.95%. This was far below the values of 11 to 20% reported by Hamoir (1955a) for nucleotropomyosin, and even below most of the values reported for tropomyosin.

In electrophoresis, this preparation exhibited a small rapid peak as already mentioned. At the end of one run, samples were withdrawn by capillary syringe from the tops of both the ascending limb and the descending limb, so as to obtain samples of the rapid material alone and of protein cleared of it. The corresponding absorptions at pH = 7 are given by Curves C and D of Fig. 9. When treated with 1 M perchloric acid, the sample containing protein exhibited very little residual absorption, while the curve of the other material was shifted, especially at the higher wavelengths (Curve E). It appeared, therefore, that cod tropomyosin extracts prepared by Bailey's procedure contained a small amount of ribonucleic acid, but that in solution at least, this and the protein existed independently.

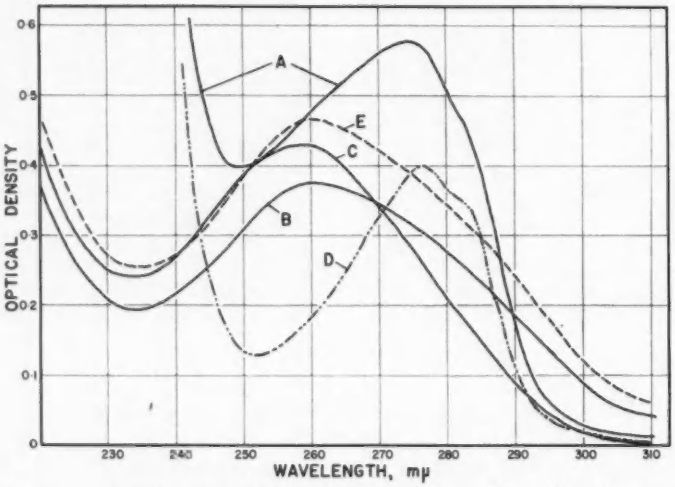

FIG. 9. Ultraviolet absorption curves of protein and nucleic acid in a preparation of cod tropomyosin.

Curve A: Unfractionated sample in 1.03 M NaCl + sodium phosphate, total $\Gamma/2 = 1.04$, pH = 6.97. Protein concentration = 0.230 mg N/ml. Curve B: Unfractionated sample after removal of protein with perchloric acid. Solvent, 0.025 M NaCl + 1.0 M $HClO_4$. Original protein concentration same as Curve A. Curve C: Sample from ascending limb after electrophoresis. Solvent: 0.10 M NaCl + sodium phosphate, total $\Gamma/2 = 0.15$, pH = 6.97. (Optical densities multiplied by $\frac{1}{2}$ to permit comparison with Curve E.) Curve D: Sample from descending limb after electrophoresis. Solvent: 1.05 M NaCl + sodium phosphate, total $\Gamma/2 = 1.08$, pH = 7.0. Protein concentration, 0.226 mg N/ml. Curve E: Sample from ascending limb after electrophoresis, after addition of equal volume of 2M $HClO_4$. Compare with Curve C.

DISCUSSION

While a direct comparison with the properties of rabbit tropomyosin is not always possible because of differences in experimental conditions, there appears to be no room for doubt that Bailey's ethanol–ether procedure for extraction of tropomyosin from muscle was succesful when applied to cod fillets. Even so, the yield (0.28 to 0.37% of muscle weight) was somewhat lower than that found by Bailey (1948) for rabbit (0.47%) or by Hamoir (1952) for carp (0.4%). It is not claimed, however, that the present extraction procedure was quantitative, and in particular, the alcohol precipitation was supected to lead to some loss of tropomyosin.

Of the proteins extractable from cod with $\Gamma/2 = 0.8$ KCl–phosphate, after prior removal of the albumins, approximately 5% was found to remain soluble at low ionic concentrations (Dingle, 1958). This amounted to approximately 0.5% of the original weight of the cod fillets and would presumably consist at least in part of tropomyosin. Similarly, in some of our unpublished work, it has been found that about 4% of such an extract remained soluble in the presence of 1.4 M ammonium sulphate, pH = 7. Tropomyosin would not be expected to precipitate under these conditions. Finally, in an ultracentrifugal investigation of salt extracts of cod, Ellis and Winchester (1959) have found that they contain a small amount of a component with a sedimentation constant of 2.1S (uncorrected). This agreed fairly well with the value found in the present work for tropomyosin. On the basis of the present results it is planned to attempt the isolation of tropomyosin from the two fractions of the salt extracts mentioned, and to determine if the component observed by Ellis was indeed tropomyosin.

SUMMARY

A protein resembling rabbit tropomyosin has been prepared from cod by means of Bailey's ethanol–ether–KCl extraction procedure. Yields were 0.28 to 0.37% of fresh weight (1.8 to 2.5% of total protein nitrogen), with an estimated purity of about 95%. Electrophoretic mobilities were measured over an ionic concentration range of 0.05 to 0.25, and a pH range of 5.9 to 7.5; they were rather strongly dependent on whether K^+ or Na^+ ions were present in the solvent. Sedimentation constants were insensitive to protein concentration except at $\Gamma/2 = 0.05$, and were in the range 2.1 to 2.4. The viscosity was lower than that reported for rabbit tropomyosin. Solubility was measured as a function of pH, at ionic concentrations of 0.10 and 1.0. The protein could be crystallized at pH 5.4 in two forms similar to those reported for other tropomyosins. The nucleic acid content of the preparation was small, and appeared to be unassociated with the protein in solution.

ACKNOWLEDGMENT

The authors are indebted to D. G. Ellis and J. T. Lauder for carrying out the ultracentrifugal measurements.

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Extractives of Fish Muscle. 2. Solvent–Water Ratio in Extraction of Fat and Water-solubles[1, 2]

By N. Dambergs

Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

The efficiency with which acetone or isopropanol can extract fat, water-solubles, and proteinaceous materials from Atlantic cod muscle depends upon changes in the water content of the solvent. Optimum dilutions for maximum yields of extractives have been determined.

INTRODUCTION

It has been shown previously that in extractions of fat from fish materials with acetone, the predominant factor in establishing yield and composition of the extract is the ratio of water to solvent (Dambergs, 1956).

The crude extract of fish muscle can be fractionated into three groups:

(a) Ethyl ether-solubles or fat.
(b) Proteinaceous materials.
(c) Non-lipid and non-protein materials, which, for the present purpose, are called water-solubles.

Each of these groups consists of a complex mixture of components. Many of the components of the lipid fraction of haddock and cod muscle have been identified (Garcia *et al.*, 1956; Lovern, 1953, 1956; Lovern and Olley, 1953a, b; Olley, 1956; Olley and Lovern, 1953, 1954). Literature concerning the non-protein nitrogenous compounds expected to be found in the water-solubles has been reviewed by Reay *et al.* (1943) and Shewan (1951). However, very little is reported about the nature and amounts of the proteinaceous extractives thus obtained. Considering the complexity of biological extracts, it is perhaps futile to attempt to reconstruct, from the results of a partial extract, the general picture of the amounts and the nature of extractives present in the original sample. However, little effort appears to have been exerted to explore the factors governing the completeness of the extraction.

The present work extends the exploration of the influence of water upon the efficiency of acetone as an extractant for the water-solubles and the proteinaceous materials. In addition, another water-miscible solvent, isopropanol, was explored in the same manner.

The determination of the maximum yields of extractives corresponding with the various solvent–water mixtures opens new avenues for a simple and rapid determination of the true amount of extractives in any fish material and enables the comparison of the extractive efficiency of any other extractant used.

[1]Received for publication August 25, 1958.

[2]An article under the title "Acetone–water mixtures for the extraction and rapid estimation of fats of biological materials, particularly fish products" that appeared in this Journal, 13 (6): 791–797, 1956, is considered as Part I of this series.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

Another objective of this exploration was to provide basic data necessary for the development of industrial processes under study at this Station in which solvent extraction is involved.

EXPERIMENTAL

To exclude the seasonal variations of the amounts of extractives and to reduce the importance of the variations in the extractives due to the origin, size, or sex of the fish, a sufficient stock of fish flesh material for the present investigation was prepared at one time by drying.

MATERIALS

Ten kg of fresh cod (*Gadus callarias*) fillets was minced and dried in a tunnel dryer, finely ground, and re-dried under reduced pressure at temperatures never exceeding 50°C. The moisture content of the freshly prepared stock material was 6.3%. It increased to 8% by the end of the experiments. The fat content was 4.1% of the dry weight and remained constant (Dambergs, 1956). The dried material was compared with the fresh starting material. The percentages of fat and water-solubles extracted before and after drying were identical. The percentages of proteinaceous materials extracted after drying were about 15% higher.

The solvents used were: acetone — reagent grade, anhydrous; isopropanol — purified, moisture 2%; ethyl ether — reagent grade, anhydrous. The solvent-water mixtures were prepared by mixing measured volumes of solvent and water.

EXTRACTION PROCEDURE

In preliminary tests it was established that for each ratio of solvent: water in the extractant mixture there is a definite maximum amount of crude extract that can be obtained from a given material and that the first four consecutive extractions of the sample yielded 95 to 98% of this amount. With isopropanol and its mixtures with water the yields of crude extract of the fifth extraction never exceeded 3% of the total amount obtained in the preceding four extractions. The yields of the sixth extraction usually were nil. Acetone containing more than 20% water gave similar results while the yields of the fifth extraction with dry acetone or acetone containing from 5 to 20% water amounted to 5% of the extractives already obtained. Samples apparently exhausted after extraction with dry acetone yielded considerable amounts of crude extract when re-extracted exhaustively with a 50% acetone–water mixture. The total yield of crude extract obtained in this manner with the two solvents was equal to the maximum amount of crude extract obtained by direct extraction of the samples with 50% acetone–water mixtures.

The following procedure was therefore adopted: 5.0-g samples were boiled under gentle reflux for 30 min with 50 ml of solvent, filtered or centrifuged, and washed with 25 ml of the same solvent, then returned to the boiling flask for the next extraction. Each sample was extracted at least four times.

DETERMINATION OF CRUDE EXTRACT

Crude extract is defined as the dry weight of material to be found in the filtrate, including washings, after evaporation of the solvent. However, it was found

that immediate drying of the crude extract complicated the subsequent determination of fat. More consistent results were obtained by defatting the crude extract first and calculating its weight by adding the dry weights of the fat and that of the defatted residue.

Determination of Fat

Fat was determined as the ethyl ether-soluble fraction of the crude extract, in the manner described by Dambergs (1956), whereby the fat was separated by washing the air-dried crude extract with 30 to 50 ml of ethyl ether in small portions, evaporating off the ether and weighing the fat after drying to constant weight.

The separation of fat from acetone extracts was easy. The ethereal solution seldom required further purification. However, the ethereal solutions of fat extracted with isopropanol were often cloudy and required purification. This was done by redissolving the thoroughly dried fat in at least 30 ml of ether. The cloudy solution obtained was cooled to a temperature near 0°C at which the white suspended particles precipitated, thus allowing their separation by decantation. Once separated, these impurities were insoluble in ether but readily soluble in water and moist ether as well as in concentrated solutions of fats in organic solvents.

The amount of impurities thus separated rarely exceeded 5% of the fat extracted with acetone but often rose to 15% for isopropanol extracts.

Determination of Protein

Protein was determined through dry weight of material precipitable by trichloracetic acid (Hoch and Vallee, 1953). The ethyl ether-insoluble fraction of the crude extract was redissolved in a measured small amount of water and quantitatively transferred into a tared 12-ml centrifuge tube. A solution of 20% trichloroacetic acid was added with stirring to 7.5% final concentration of the reagent. After centrifuging and decantation, the precipitate was washed in the tube with a small amount of 7.5% trichloroacetic acid solution and dried to constant weight at 110°C.

Determination of Water-soluble Materials

The water-solubles were determined by evaporating the combined supernates and washings from the protein determination. The major part of the trichloroacetic acid should be decomposed by diluting and boiling for at least an hour before the evaporation. If this precaution is not taken more than 10% of the water-solubles may be lost as volatile products during the last stage of evaporation. In practice these losses occur only with the first two extractions of each sample. The results can be checked by calculating the difference between the ethyl ether-insoluble materials and the protein.

RESULTS

Acetone–Water

Figure 1 shows, as percentage of dry sample weight, the total amounts of crude extract, water-solubles, protein, and fat obtained through four extractions of a sample of the dried cod flesh material by acetone containing various amounts of water.

The amount of crude extract increased steadily with dilution of the acetone. The range was from 3.9% for dry solvent to 32.7% for the most dilute acetone used (85% water). Water only, in four consecutive extractions, yielded 35.4%. Some water-soluble materials were present even in extracts obtained with dry acetone. The amount increased gradually with dilution of the solvent and reached a maximum of 15.6%, or 3000 mg per 100 g of fresh fillet, with acetone extractants containing 40 to 85% water. These values were checked by extracting exhaustively various samples of fresh cod fillets, adjusting the amount of acetone used for the

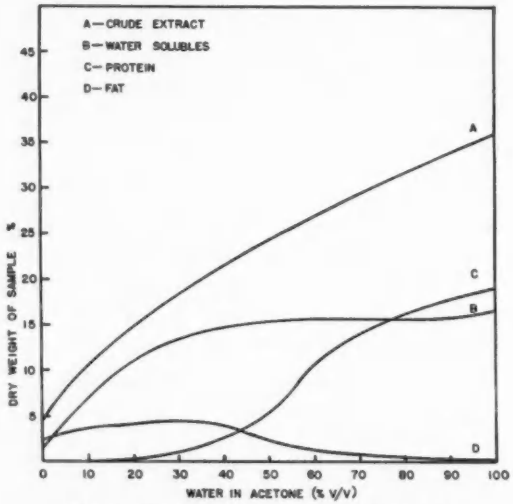

FIG. 1. Yields of extractives obtained with acetone–water mixtures.

first extraction to have 30% water in the liquid phase, and using acetone with 30% added water for the following extractions. The amounts of water-soluble materials extracted in the first four extractions of fresh fillets were practically identical with those obtained from dried material. The deviations did not exceed $\pm 2.5\%$.

There was a further increase of extracted water-soluble materials when boiling water was used as the extractant. However, this increase appeared to be due to a breakdown of the water-insoluble proteins. The amounts found after the fifth extraction were constant and were composed of approximately 15% water-solubles and 85% protein, a much higher protein content than in the acetone extracts.

Using acetone containing from 0 to 20% water there were small but measurable amounts of proteinaceous materials which precipitated with trichloroacetic acid in all the crude extracts. The amounts increased gradually from 0.1% for dry acetone to 0.4% for acetone containing 20% water. With more diluted acetone the amount of the protein extracted increased gradually and reached a value of 17.0% in four extractions with acetone containing 85% water. With

acetone–water mixtures the fifth extraction yielded small amounts of proteinaceous materials. However, there were no apparent signs of hydrolysis of the unextractable proteins. By extrapolating the results obtained in five consecutive extractions with acetone containing 85% water, the "true" value for extractable proteinaceous materials of 3300 mg per 100 g of fresh cod muscle was obtained. Exhaustive extraction of fresh fillets with acetone was carried out only with acetone containing 30% water whereby the results were 25% lower than when the dried material was similarly extracted.

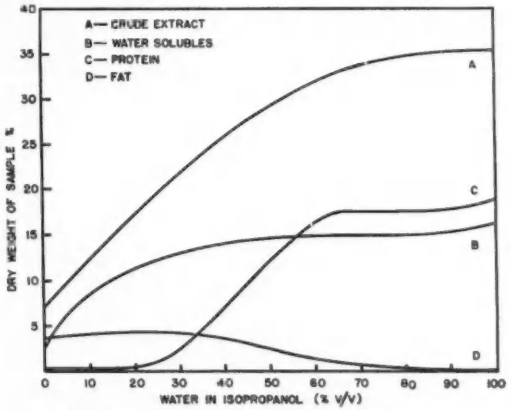

FIG. 2. Yields of extractives obtained with isopropanol–water mixtures.

The extracted fat increased gradually with increasing dilution of the acetone to a maximum of 30% water, and then decreased gradually at higher water contents.

ISOPROPANOL–WATER

Results obtained with isopropanol–water mixtures are shown in Fig. 2. When comparing these with the results shown in Fig. 1, it is seen that in the absence of water the amount of crude extract obtained with isopropanol was slightly higher than with acetone. This increase was due mainly to the larger amounts of fat and also water-solubles extracted. The increase in amounts of fat extracted with the dilution of isopropanol was not as sharp as with acetone. However, here again maximum extraction was achieved with diluted solvent, containing about 22% water. The decrease of the Curve D after this point was not as steep as in the case of acetone.

The maximum amount of water-solubles extracted (Curve B) was reached at the same dilution as with acetone, i.e. at 55 to 85% water in the solvent, but the maximum amount was about 2.5% lower than with acetone.

The protein extracted reached a maximum of 17.4% for isopropanol containing 65 and 85% water. This value was the same as the extrapolated value for acetone containing 85% water.

DISCUSSION

The efficiency of a solvent may be indicated by the ratio of the amount of material extracted to the true amount of this material present in a given sample. For practical application it is desirable that the maximum amount be extracted in the shortest possible time.

For the materials used in this study, the "true" amounts of extractives found are given in Table I.

TABLE I. "True" amounts of extractives found in cod muscle.

Extractive	Content	
	g/100 g (dry basis)	mg/100 g (wet basis)
Fat	4.1	800
Water-solubles	15.2	3000
Proteinaceous material	17.4	3300
Totals	36.7	7100

According to these results the cod muscle contains: 11.9% non-extractable protein, 3.3% extractable protein, 3.0% water-solubles, 0.8% fat, and 81% water (by difference). Some of the extracted residues were checked for their ash content. They still contained about 15% of the initial ash. The extracted ash was mainly concentrated in the water-solubles. These values are in close agreement with the average data found for cod by other investigators (Shewan and Jones, 1957).

The average "true" fat content of cod muscle, dried or fresh, as determined by alternate extraction with dry and wet acetone (Dambergs, 1956) on more than 30 different samples was never lower than 3.95% or higher than 4.20% of dry weight, and 800 mg ($\pm 3\%$) per 100 g of fresh fillets. The main reason why greater precision was not attained is the difficulty of determining more precisely the moisture content of the sample.

Only a part of the true fat can be extracted with dry acetone or dry isopropanol. Since both solvents are powerful dehydrants, the amount of fat extracted from a wet sample will depend upon the ratio of the solvent to the amount of water present in the sample. Maximum yields are obtained by adjusting this ratio between 20 to 30% water in the liquid phase.

The "true" values of the water-solubles were remarkably constant for both fresh and dried materials. The yields seemed to increase slightly with age of the undried material, but more analyses are necessary to draw firm conclusions.

The precision of the determination of the proteinaceous material rests mainly upon the technique used. The method used was very satisfactory with extracts containing amounts of protein not exceeding 5 mg per ml but the agreement between results of duplicates diminished rapidly for extracts with more than 10 mg per ml. In several cases final concentrations of 5% and more than 10% trichloroacetic acid gave results lower than concentrations between these values. Hence the concentration of 7.5% was used.

The extractive power of acetone versus isopropanol is directly compared by the curves in Fig. 3, 4, and 5, which represent values obtained by expressing the

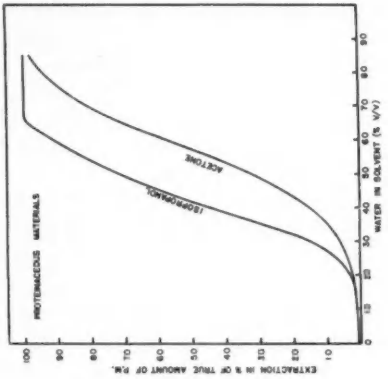

FIG. 5.

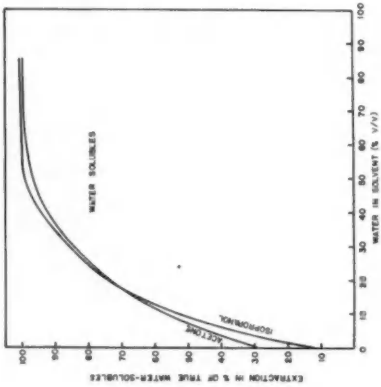

FIG. 4.

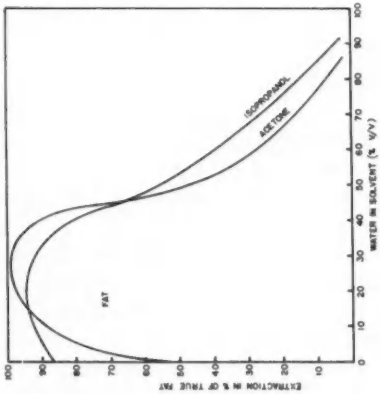

FIG. 3.

FIG. 3, 4, 5. Extractive efficiencies of acetone and isopropanol in mixtures with water.

yields of each solvent–water mixture as a percentage of the "true" amounts of materials given in Table I.

In the absence of water the efficiency of isopropanol as an extractant of the fat from cod muscle was higher than that of acetone (Fig. 3). The efficiency of both solvents initially increased with increasing dilution. This increase was much steeper for acetone. The maximum of efficiency for both solvents was in the same region of dilution and beyond this it decreased sharply.

Dry acetone extracted only 10% of the water-solubles whereas dry isopropanol extracted 30% (Fig. 4). The efficiency of both solvents initially increased sharply with dilution, and reached a maximum at about the same dilution (50% water).

The efficiencies of the dry solvents in extracting proteinaceous materials were low and changed but little with dilution up to the point where the solvent contained 20% water (Fig. 5). At greater dilutions isopropanol was more efficient as an extractant of proteinaceous materials than was acetone with similar water content.

CONCLUSION

The predominant factor regulating the yields of fat, water-solubles and proteinaceous materials in extractions of cod muscle with acetone or isopropanol is the ratio of water to solvent.

With each ratio of water to solvent there is a corresponding maximum yield of crude extract. The composition of the crude extract varies continuously with the dilution of the solvent. For each group of extractives there is an optimum zone of dilution where maximum extraction is possible. For acetone and isopropanol these dilutions are:

(a) Solvent containing from 20 to 30% water achieves maximum extraction of fat.

(b) Solvent containing at least 45% water is required for maximum extraction of the water-solubles.

(c) Isopropanol containing at least 60% water or acetone containing 85% water achieves maximum extraction of proteinaceous materials.

A selective maximum extraction of each of the three groups of extractives with a minimum extraction of the two other groups of materials is possible by properly adjusting the solvent-to-water ratio, or the alternate use of various solvent–water mixtures.

The efficiency of dry isopropanol in extracting fat is less affected by the addition of water than is the efficiency of acetone. However, the fat extracted with isopropanol requires more thorough purification than the fat obtained with acetone.

The "true" amounts of fat, water-solubles and proteinaceous materials in a sample of cod muscle have been established. For both solvents, within the limits of experimental errors, the values are identical regardless of whether acetone or isopropanol is used as the extractant.

However, only after a complete qualitative exploration of the extracts will it be possible to conclude whether these amounts were originally present in the

fresh cod muscle or whether their quantities and qualities have changed during the extraction or the preparation of the sample.

The present results have found an immediate practical application in the solution of the technical problems occurring in the production of defatted concentrates of fish protein.

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Errors in Estimates of Mortality Obtained from Virtual Populations[1]

By Yvonne M. M. Bishop[2]

Inter-American Tropical Tuna Commission, La Jolla, California

ABSTRACT

The bias in individual estimates of the natural mortality coefficient derived from the ratio of successive virtual populations is defined algebraically and is shown to be unchanged whether one or more year-classes is considered, if the mortality coefficients are assumed to be constant for all exploitable fish. Limiting and probable values of this bias are shown graphically for a coefficient of fishing mortality ranging from 0 to –2.0 in the year for which the estimate is obtained. These values are drawn for a true natural mortality of –0.2 and –0.4 and for both an increasing and a decreasing fishing effort. Bias in individual estimates of natural mortality is greatest when there are large fluctuations in fishing effort, particularly when fishing mortality is low relative to natural mortality, and it increases with increased natural mortality.

A linear regression of a series of virtual population ratios would in general give an intercept value which underestimated the coefficient of natural mortality, and a slope which overestimated the coefficient of fishing mortality, in situations where F has tended to increase and also where it has had no trend (the "steady state" of Table I). Both these errors would be in the opposite direction during a period when there was a decline in fishing effort.

INTRODUCTION

Virtual populations have been used to estimate the total mortality rate, and hence the survival rate, of a fish population from knowledge of the annual catch of each age-group in the fishery and the relative annual fishing effort. This method has been claimed by Fry (1957) to offer the advantages that "no iteration appears necessary in the computations to compensate for differences in fishing effort in the year for which the survival is estimated" and secondly "that the index of survival based on the virtual population is not so sensitive to a drift in the vulnerability of the fish to the fishery". He compares results obtained from this method with those obtained by Beverton (1954) using an iterative method to analyze data on Opeongo Lake trout covering a ten-year period during which the fishing effort decreased gradually from 22.4 to 5.7 and then increased gradually to 17.4 (unit of measurement was hundreds of boat-hours). Fry's estimate of c, the coefficient of fishing mortality, and of M, the natural mortality, are –0.0262 and –0.0818 respectively, compared with c of –0.0260 and M of –0.1000 obtained by Beverton's method.

At a conference held in Lisbon in June, 1957, sponsored by ICES, FAO, and

[1]Received for publication April 11, 1958.

[2]Present address: c/o St. Paul's Rehabilitation Centre for the Blind, 770 Centre Street, Newton Centre 58, Massachusetts.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

ICNAF, a workshop meeting on the treatment of data considered the use of virtual populations for estimating mortality. Some tentative conclusions were drawn, of which the second was as follows: "If natural mortality is constant but fishing mortality varies from year to year due to changes in effective fishing effort then the use of the 'virtual population' will tend to exaggerate changes in total mortality and give too low an intercept value of natural mortality in a plot of total mortality against effort. This bias will be greater, the greater are the variations in fishing mortality and the lower is its value relative to the natural mortality". The suggestion of a low intercept of natural mortality seems to be borne out by Fry's example quoted above, although in this particular case the difference between the results obtained by this method and Beverton's is small.

Since the virtual population method necessarily assumes a constant natural mortality and is only applicable when fishing effort changes from year to year, it seemed to be desirable to define more closely the circumstances under which a biased estimate of natural mortality rate occurs and to obtain an idea of how great the error might be.

VIRTUAL POPULATION IN TERMS OF FISHING AND NATURAL MORTALITY PARAMETERS

It is first necessary to express virtual populations in terms of fishing and mortality coefficients. The usual notation has been adopted, *i.e.*

M_r = natural mortality in year r

F_r = fishing mortality in year r

$= cf_r$ where c is constant and f_r is a measure of the fishing effort in year r.

It is assumed that fishing and natural mortality do not vary with the age of the fish but that fish of age "a" or more are equally vulnerable. If the number of recruits of age a are R_1, R_2, R_3 . . . etc. in years, 1, 2, 3 . . . etc., then, assuming each age group decreases exponentially due to fishing and natural mortality, the numbers of each age-group present at the beginning of each year may be written thus

	Age a	Age b	Age c
Year 1	R_1		
Year 2	R_2	$R_1e^{F_1+M_1}$	
Year 3	R_3	$R_2e^{F_2+M_2}$	$R_1e^{F_1+M_1+F_2+M_2}$
Year 4	R_4	$R_3e^{F_3+M_3}$	$R_2e^{F_2+M_2+F_3+M_3}$

It is apparent that this process of adding coefficients for each year of life in the fishery can be continued for any number of years until the age-group is extinct. It should be noted that, in accordance with the notation used by Fry, F_r and M_r are always negative. Although the method of obtaining mortality rates from virtual populations requires that M be constant for all ages and years the general expressions have been written with suffixes for each year because, as will be shown later, different estimates of M are obtained for each F_r.

By considering the number of each year-class present each year the catch from each age-group during each year may be written

	Age a	Age b	Age c
Year 1	$\frac{F_1R_1(1-e^{F_1+M_1})}{F_1+M_1}$		
Year 2	$\frac{F_2R_2(1-e^{F_2+M_2})}{F_2+M_2}$	$\frac{F_2R_1e^{F_1+M_1}(1-e^{F_2+M_2})}{F_2+M_2}$	
Year 3	$\frac{F_3R_3(1-e^{F_3+M_3})}{F_3+M_3}$	$\frac{F_3R_2e^{F_2+M_2}(1-e^{F_3+M_3})}{F_3+M_3}$	$\frac{F_3R_1e^{F_1+M_1+F_2+M_2}(1-e^{F_3+M_3})}{F_3+M_3}$

Now letting $\frac{F_r(1-e^{F_r+M_r})}{F_r+M_r} = X_r$ for all values of r, the catch during each year may be written more simply

	Age a	Age b	Age c
Year 1	R_1X_1		
Year 2	R_2X_2	$R_1X_2e^{F_1+M_1}$	
Year 3	R_3X_3	$R_2X_3e^{F_2+M_2}$	$R_1X_3e^{F_1+M_1+F_2+M_2}$

The virtual population, as defined by Fry, is "the sum of the fish, belonging to a given year-class, present in the water at any given time that are destined to be captured in the fishery in that year and all subsequent years". Thus the virtual population of age a in year 1 is

$$V_{a1} = R_1 (X_1 + X_2e^{F_1+M_1} + X_3e^{F_1+M_1+F_2+M_2} + \ldots)$$

sufficient terms being taken to account for all years until the year class becomes extinct in the fishery. Now let

$$k_1 = X_2 + X_3 e^{F_2+M_2} + X_4 e^{F_2+M_2+F_3+M_3} + \ldots$$

i.e. k_1 = terms which do not contain F_1 or M_1

then
$$V_{a1} = R_1 (X_1 + e^{F_1+M_1}k_1) \quad (1)$$

and similarly
$$V_{b2} = R_1 e^{F_1+M_1}k_1 \quad (2)$$

It may also be noted that, since k_1 is equal to the fraction of the brood caught in all years after the first, k_1 is a positive integer less than or equal to 1.

METHOD OF OBTAINING NATURAL MORTALITY FROM VIRTUAL POPULATIONS

The method of estimating the natural mortality, M, and the coefficient of fishing mortality, c, consists of plotting the logarithm of z_r against f_r for all values of r available, where z_r is a function of the virtual population. A linear regression then gives an estimate of M as the intercept on the log-z axis and c as the slope of the line. In other words $\log_e(z_r)$ is taken as an estimate of $M_r + cf_r$, or in the simpler notation as an estimate of $M_r + F_r$.[2]

[2]In all subsequent references to logarithms the base e is assumed. In practice the base 10 is sometimes used and appropriate adjustments made later.

According to the data available z_r is derived from a single year-class or from two or more year-classes. Taking first the single year-class, z_r is simply the inverse ratio of the virtual population of fish of age a in the first two successive years in the fishery. Thus

$$z_1 = \frac{V_{b2}}{V_{a1}}$$

and therefore from equations (1) and (2) above

$$z_1 = \frac{e^{F_1+M_1} k_1}{X_1+e^{F_1+M_1} k_1} \tag{3}$$

or, in general, taking logarithms

$$\log (z_r) = F_r+M_r+\log \left\{ \frac{k_r}{(X_r+e^{F_r+M_r} k_r} \right\} \tag{4}$$

and since $\log(z_r)$ is taken as an estimate of $F_r + M_r$, the bias introduced is the logarithm on the right-hand side of equation (4).

If z_r is derived from more than a single year-class it is necessary to define the virtual population of successive year-classes for the years under consideration. Taking for simplicity two broods in years 1 and 2, the additional virtual populations required for definition of z'_r are V_{b1} and V_{c2}.

By analogy with V_{a1} it may be seen that

$$\begin{aligned} V_{b_1} &= R_0 e^{F_0+M_0} (X_1+X_2 e^{F_1+M_1}+X_3 e^{F_1+M_1+F_2+M_2}+\ldots) \\ &= R_0 e^{F_0+M_0} (X_1+e^{F_1+M_1} k_1) \end{aligned}$$

This assumes that the same number of terms are required before the two virtual populations V_{a1} and V_{b1} are extinct, which would mean that the fish from V_{b1} attained an age one year greater than those from V_{a1} an unlikely assumption. As in practice it is not possible to obtain reliable estimates of the numbers of very old fish in the catch and an approximation is often used, the assumption is nevertheless retained. By a similar comparison with V_{b2} it may be seen that

$$V_{c_2} = R_0 e^{F_0+M_0} (e^{F_1+M_1} k_1)$$

Then the ratio to be plotted is

$$\begin{aligned} z'_1 = \frac{V_{b2}+V_{c2}}{V_{a1}+V_{b1}} &= \frac{R_1(e^{F_1+M_1} k_1)+R_0 e^{F_0+M_0} (e^{F_1+M_1} k_1)}{R_1 (X_1+e^{F_1+M_1} k_1)+R_0 e^{F_0+M_0} (X_1+e^{F_1+M_1} k_1)} \\ &= \frac{e^{F_1+M_1} k_1 (R_1+R_0 e^{F_0+M_0})}{(X_1+e^{F_1+M_1} k_1) (R_1+R_0 e^{F_0+M_0})} \\ &= \frac{e^{F_1+M_1} k_1}{X_1+e^{F_1+M_1} k_1} \\ &= \frac{V_{b2}}{V_{a1}} \quad \text{as given in equation (3) for the definition of } z_1. \end{aligned}$$

Thus $z' = z$ and it is apparent that the addition of further year-classes will not alter the definition of the ratio; the use of more than one year-class may reduce the random error but it will not affect the bias as defined in (4) above.

RELATIONSHIP BETWEEN BIAS AND MORTALITY RATES

The value of c obtained by plotting log (z_r) against f_r depends on the units of measurements of f_r. The bias involved in the method is obviously not affected by units of measurement and it is therefore permissible to consider the results of plotting $\log(z_r)$ against F_r, *i.e.* taking $c = 1$. If there were no bias the points for various F_r would be randomly distributed about the straight line at 45° to the F-axis that cuts the $\log(z)$-axis at the true value of M. It is also apparent that if $\log(z_r) - F_r$ were plotted against F_r, the points would, in the absence of bias, be randomly distributed about the line which is parallel to the F-axis and distant from it by the amount M. (If there were no random error they would of course lie on this line.)

Since we have seen in equation (4) that the bias does exist, let the biased estimate of M for the year r be denoted by $\hat{M}_r$. Then

$$\hat{M}_r = \log(z_r) - F_r = M_r + \text{bias in year } r.$$

It is proposed to illustrate graphically the relationship between $\hat{M}_r$ and F_r under the effect of different fishing and mortality rates and hence the error in M*, the estimate of M obtained by the virtual population method, may be deduced.

It is necessary first to consider the maximum and minimum values of $\hat{M}_r$ that can be obtained for a specific M for each value of F_r. This may be done by differentiating $\hat{M}_r$ with respect to F_r. Since $\hat{M}_r = \log(z_r) - F_r$, dropping suffixes, which are r in every case, and differentiating

$$\frac{d\hat{M}}{dF} = \frac{1}{z} \cdot \frac{dz}{dF} - 1 \tag{5}$$

but

$$z = \frac{ke^{F+M}}{X + ke^{F+M}}$$

and thus since k_r is independent of F_r

$$\frac{d(z)}{dF} = \frac{ke^{F+M}\left\{X - \frac{dX}{dF}\right\}}{(X + ke^{F+M})^2}$$

substituting in (5) gives

$$\frac{d(\hat{M})}{dF} = \frac{X - \frac{dX}{dF}}{X + ke^{F+M}} - 1 \tag{6}$$

but by definition

$$X = \frac{F}{F+M}(1 - e^{F+M})$$

$$= \frac{Fp}{q}$$

where $p = 1-e^{F+M}$ and $\frac{d(p)}{dF} = -e^{F+M} = p-1$

$$q = F+M \text{ and } \frac{d(q)}{dF} = 1$$

Then $$\frac{d(X)}{dF} = \frac{q\{F(p-1)+p\}-Fp}{q^2}$$

and substituting in (6) with new terminology

$$\frac{d(\hat{M})}{dF} = \frac{Fq+Fp-qp}{Fpq+kq^2(1-p)} - 1$$

for a maximum or minimum value of $\hat{M}$, $\frac{d(\hat{M})}{dF} = 0$ and so

$$\frac{Fq+Fp-qp}{Fpq+kq^2(1-p)} = 1 \text{ or } F = \frac{qp+kq^2(1-p)}{q+p-pq} = \tilde{F} \text{ say}$$

which again may be written

$$\frac{\tilde{F}}{q} = 1+\frac{q(1-p)(k-1)}{p+q(1-p)}$$

but $$\frac{\tilde{F}}{q} = \frac{\tilde{F}}{\tilde{F}+M}$$ which is positive and less than 1.

Therefore $$\frac{q(1-p)}{p+q(1-p)}(k-1)$$ must be negative

but it may be shown that, since $q = F + M$ which is negative and $p = 1 - e^{F+M}$ which is positive, k must be greater than 1 for $\tilde{F}$ to have a negative value. Therefore for $k \leqslant 1$ there is no maximum or minimum value of $\hat{M}$. It may also be shown that, when $F = 0$ and hence $q = M$,

$$\frac{d\hat{M}}{DF} = \frac{-p}{kM(1-p)} - 1 \text{ which is always positive for negative M.}$$

Thus if $\frac{d\hat{M}}{dF}$ is positive when $F = 0$ and for $k \leqslant 1$ is never zero, it may be deduced, since it is a continuous function, that $\frac{d\hat{M}}{dF}$ is always positive for $k \leqslant 1$, for all negative F.

Thus for a given value of M, there is a family of curves for $0 \leqslant k \leqslant 1$ relating $\hat{M}$ and F such that $\hat{M}$ increases as F increases. These are drawn, in Figures 1 and 2 for $M = -0.2$ and in Figures 3 and 4 for $M = -0.4$, for 0.1 intervals of k between 0 and 1 (Feint lines). It is known that k is a positive number and less than or equal to 1, but it is now necessary to consider what values it can take under given circumstances; that is, to define its value when there is no bias, and its value when F does not change, and also the relationship between successive k's, and hence define the bias in M in terms of k only. To do this it will be necessary to reintroduce suffixes.

VALUES OF k

(1) VALUE WHEN $\hat{M} = M$

From equation (4) it may be seen that if $\hat{M}$ is an unbiased estimate of M then

$$\log\left[\frac{k_r}{X_r+e^{F_r+M_r}k_r}\right] = 0, \text{ where } X_r = \frac{F_r}{F_r+M_r}(1-e^{F_r+M_r}) \text{ by definition}$$

and hence

$$k_r = \frac{F_r}{F_r+M_r}$$

(2) VALUE WHEN F IS CONSTANT, AFTER AN INITIAL CHANGE

If after a change from F_r in year r to F_{1+r} in year $r+1$ the fishing mortality remains constant, then $X_{r+1} = X_{r+2} = X_{r+3}$ etc. and

so $$k_r = X_{r+1}(1+e^{F_{r+1}+M_{r+1}}+e^{2(F_{r+1}+M_{r+1})}+e^{3(F_{r+1}+M_{r+1})}+\ldots \text{ for n}$$

terms say, and summing

$$k_r = \frac{X_{r+1}(1-e^{(n+1)(F_{r+1}+M_{r+1})})}{1-e^{F_{r+1}+M_{r+1}}}$$

and substituting for X_{r+1}

$$k_r = \frac{F_{r+1}}{F_{r+1}+M_{r+1}}(1-e^{(n+1)(F_{r+1}+M_{r+1})})$$

and as n approaches infinity, $e^{(n+1)(F_{r+1}+M_{r+1})}$ approaches zero and so

$$k_r = \frac{F_{r+1}}{F_{r+1}+M_{r+1}}$$

By comparison with the expression for k obtained above when there is no bias it is apparent that M_r represents the true value of M when there is no change at all in the fishing effort for year r and all subsequent years.

(3) RELATION BETWEEN SUCCESSIVE k's

By definition

$$k_1 = X_2+e^{F_2+M_2}X_3+e^{F_2+M_2+F_3+M_3}X_4+\ldots$$
$$= X_2+e^{F_2+M_2}(k_2) \text{ where } k_2 \text{ is independent of } F_2.$$

By analogy

$$k_r = X_{r+1}+e^{F_{r+1}+M_{r+1}}k_{r+1}$$

(4) BIAS OF ESTIMATE IN TERMS OF k

The bias of M_r as an estimate of M has been previously defined as

$$\log\left[\frac{k_r}{X_r+e^{F_r+M_r}k_r}\right]$$

and it may be seen from the relation of successive k's that this is the same as

$$\log\left[\frac{k_r}{k_{r-1}}\right]$$

Using this knowledge of the variations in the values of k it is now possible to relate values of $\hat{M}_r$ to the families of curves of k previously drawn.

PLOTTING EFFECT OF SPECIFIC CHANGES IN F

In order to superimpose the values of $\hat{M}_r$ that would be obtained following specific changes in fishing effort it is necessary to classify the possible changes that may occur. If the fishing changes between the year r and the following year by an amount D such that

$$F_{r+1} - F_r = D$$

then in the third year, $r + 2$, it may (a) remain the same, (b) decrease or (c) increase.

It has been shown (page 79) that in case (a) $k_r = \dfrac{F_{r+1}}{F_{r+1}+M_{r+1}}$, if the fishing remains steady after the initial change.

For the other two cases it is necessary to consider the relationship between successive k's. We have from above that

$$k_r = X_{r+1} + e^{F_{r+1}+M_{r+1}} k_{r+1}$$

If the fishing stops in the third year and is not resumed *i.e.* $F_{r+2} = F_{r+3} = \ldots = 0$ then $k_{r+1} = 0$ and $k_r = X_{r+1}$. This is an extreme example of case (b) and gives the lowest possible value for k_r. Any other example of case (b) would give values of k_r between this limiting value and the value obtained for case (a). Similarly if in the third and subsequent years the fishing becomes extremely heavy, then k_{r+1} approaches the value 1. Thus for case (c) the limiting value of k_r is $X_{r+1} + e^{F_{r+1}+M_{r+1}}$.

The values of k are thus for

case (a) $k_r = \dfrac{F_{r+1}}{F_{r+1}+M_{r+1}}$ — corresponding to steady fishing after the initial change, D, from F_r to F_{r+1}

case (b) (limiting value) $k_r = X_{r+1} = \dfrac{F_{r+1}}{F_{r+1}+M_{r+1}}(1-e^{F_{r+1}+M_{r+1}})$ — the smallest possible value of k_r, obtained when the change D is followed by the greatest possible drop in the third year *i.e.* cessation of fishing

case (c) (limiting value) $k_r = X_{r+1} + e^{F_{r+1}+M_{r+1}} = \dfrac{F_{r+1}}{F_{r+1}+M_{r+1}} + \dfrac{M_{r+1}}{F_{r+1}+M_{r+1}} e^{F_{r+1}+M_{r+1}}$ — the largest possible value of k_r, obtained by the greatest possible increase in the third year, *i.e.* all remaining fish caught at the beginning of the third year before any died naturally

These three curves for k_r are plotted for $M = -0.2$ and $M = -0.4$ in Figures 5 and 6 respectively; they were obtained by computing k for selected values of F_{r+1}. Also drawn is the curve obtained when the values of k are computed for a fishery in which F_r increases regularly by 0.1 each year, curve (d); it is seen that such a gradual increase does not give values very different from that obtained with constant F, curve (a). From these curves the values of F_{r+1} corresponding to selected values of k are obtained, i.e. for $k = 0.1$, 0.2 etc. These values of F_{r+1} are tabulated and then by adding or subtracting $|D|$ the values of F are obtained for either a decrease or increase in fishing between years r and $r + 1$.

Increased Fishing Effort[3]

Since F is negative, for an increase in fishing effort the equation $F_{r+1} - F_r = D$ gives negative values of D. The selected values of D were 0.1 and 0.2 and these were subtracted from the values of F_{r+1} in cases (a) (the steady state) and (b) (the sudden drop). The resultant values of F_r are superimposed on Figures 1 and 3, where the heavy lines thus represent the values of M_r obtained for each F_r under given conditions. The asymptote when $D = 0$ is also added, which of course corresponds to the line $\hat{M}_r = M$ when there is no subsequent change in fishing effort. When D is infinitely large the limiting values of $\hat{M}$ given by the line $k = 1.0$ are obtained. The values obtained when the fishing is increased by 0.1 each year are shown by a dotted line.

These superimposed lines show whether $\hat{M}_r$ is an underestimate or overestimate of M, and whether the bias $|\hat{M}_r| - |M|$ increases or decreases with changes in F, D and M. For convenience the notation $E = |\hat{M}_r - M|$ is used, thus E is always a positive number. It will be seen that for an increase of fishing followed by a "steady state" the value of $\hat{M}_r$ is always an underestimate of M and that E increases as |D| increases, but that for very large initial fishing effort the value of $\hat{M}_r$ is very close to M, *i.e.* E is small. An increase followed by a drastic reduction in fishing can on the other hand give an overestimate of M much greater than any possible underestimate and this overestimate is inversely related to the magnitude of D; these overestimates also decrease as F_r increases but are still considerable even for large F_r. The values obtained when the fishing increases by 0.1 each year are very close to those obtained when the fishing increases by 0.1 in the second year and then remains steady. Thus it may be seen that during a period when the fishing is increasing, either abruptly or gradually, underestimates of M are obtained, but when this is followed by a period when fishing is drastically curtailed, as happened to some fisheries during the war, overestimates of M are obtained.

From comparison of Figures 1 and 3 it is concluded that for M between −0.2 and −0.4 in case (a) the steady state, E increases slightly with M, particularly for low F_r, whereas for case (b) E decreases considerably as M increases throughout the range of F_r drawn, *i.e.* 0 to 2.0. The lower limit of $\hat{M}_r$ given by $k = 0$, gives a much greater E for $M = -0.4$ than for $M = -0.2$. Thus for any specific negative D and F_r the range of possible values of $\hat{M}_r$ is increased by an increase in M but the line $\hat{M}_r = M$ lies more nearly in the center of the range for the larger M. Effects of changes in M outside the range −0.2 to −0.4 have not been investigated as they are not so frequently encountered. These results and those for an initial decrease in fishing effort have been summarized in Table I.

Decreased Fishing Effort

The corresponding effects of a decrease in fishing effort between years r and $r+1$ are shown in Figures 2 and 4. For case (a), when the fishing is steady following the initial drop, an overestimate of M is now obtained; this overestimate

[3]In what follows (including Table I) the fact that $\hat{M}_r$ is actually a negative value has been ignored—*e.g.*, "$\hat{M}_r$ is an overestimate of M" means that $|\hat{M}_r| > |M|$.

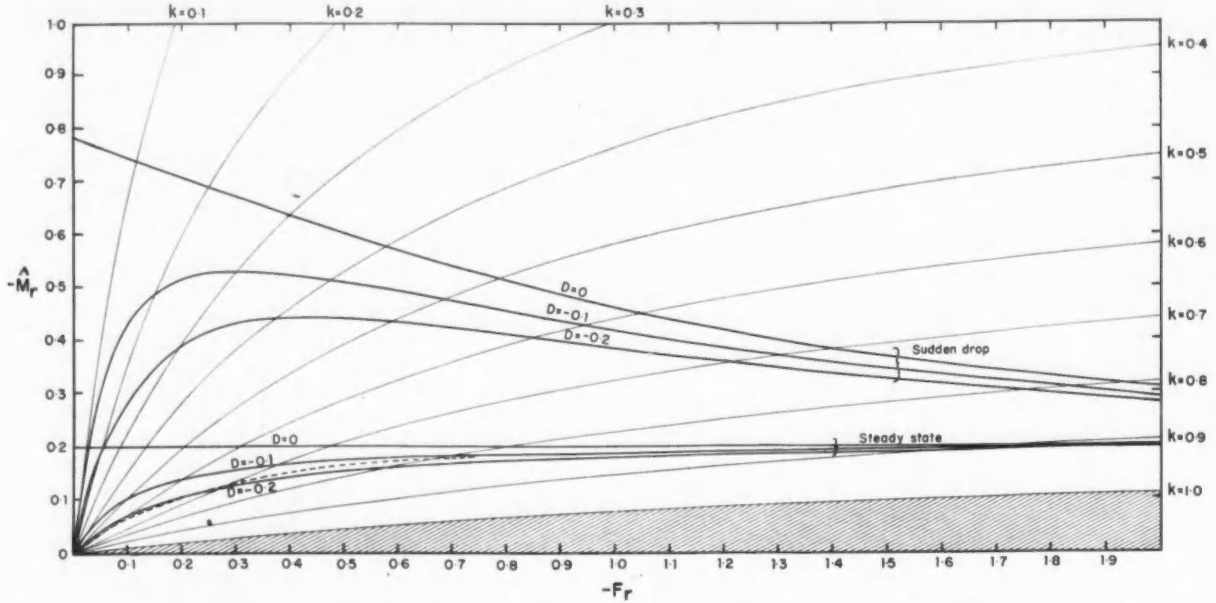

FIG. 1. Estimates of mortality rate obtained when the true mortality rate, M, is –0.2 and the fishing *increases* between the first and second year by different amounts, *i.e.* for D = 0, –0.1, –0.2, where $F_{r+1} - F_r = D$.
Heavy lines indicate the values of $\hat{M}_r$ obtained for a given F_r when
(a) a "steady state" is reached, *i.e.* fishing in the second and subsequent years is the same, $F_{r+1} = F_{r+2}$ etc. (b) there is a sudden drop in fishing effort, *i.e.* fishing ceases in the third and subsequent years, $F_{r+2} = F_{r+3} = \ldots = 0$. Values obtained when the fishing effort continues to increase will lie between those for the "steady state" and the curve $k = 1.0$. Dotted line shows the effect of an increase in fishing mortality of 0.1 each year.

increase in fishing mortality of 0.1 each year.

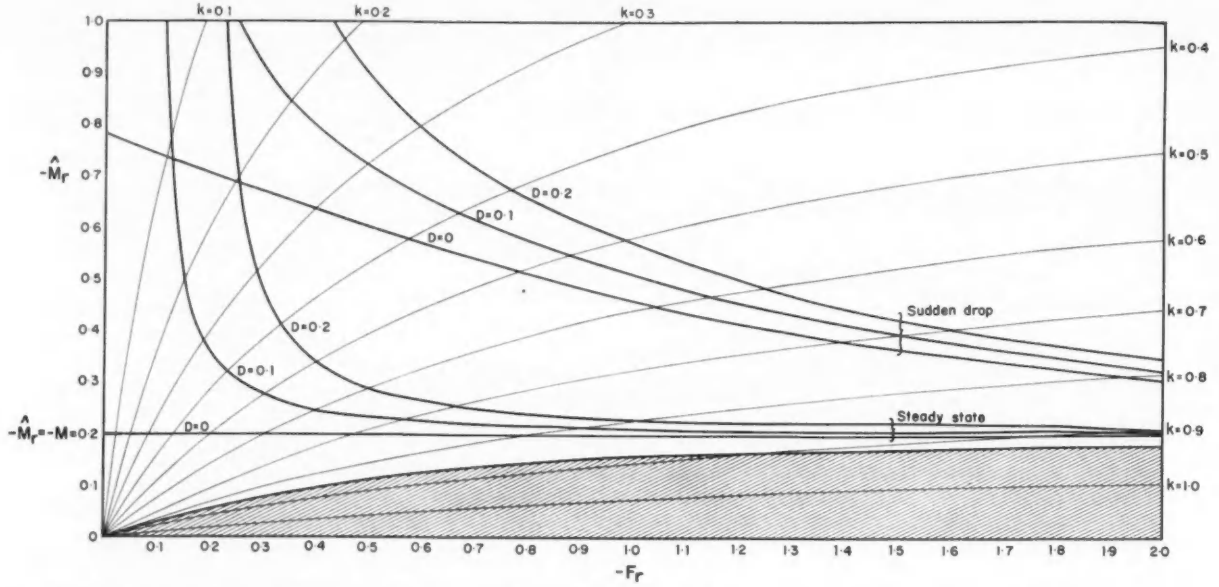

FIG. 2. Estimates of the mortality rate obtained when the true mortality rate, M, is –0.2 and the fishing mortality *decreases* in the second year by different amounts, *i.e.* for $D = 0$, 0.1, 0.2 where $F_{r+1} - F_r = D$.
Heavy lines indicate the values of $\hat{M}_r$ obtained for a given F_r when
(a) a "steady state" is reached, *i.e.* fishing intensity in the second and subsequent years is the same, $F_{r+1} = F_{r+2}$ etc. (b) there is a sudden drop in fishing effort, *i.e.* fishing ceases in the third and subsequent years, $F_{r+2} = F_{r+3} = \ldots = 0$. Values obtained when the initial drop in fishing intensity is followed by an increase will lie between those for the "steady state" and the shaded area.

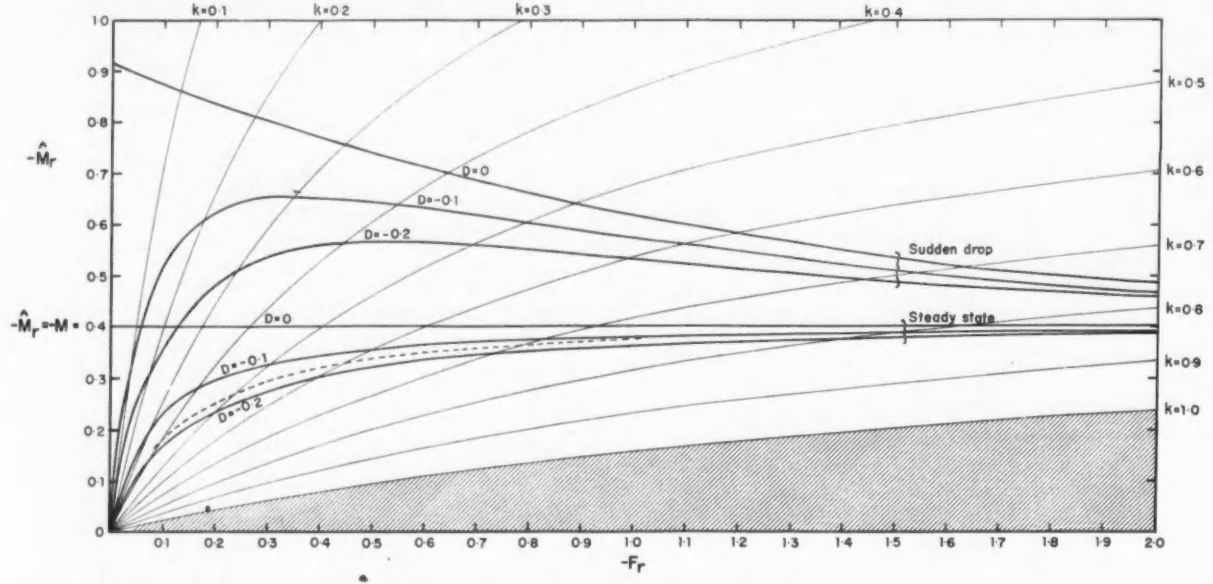

FIG 3. Estimates of mortality rate obtained when the true mortality rate, M, is –0.4 and the fishing *increases* between the first and second year by different amounts, *i.e.* for $D = 0, -0.1, -0.2$ where $F_{r+1} - F_r = D$.
Heavy lines indicate the values of $\hat{M}_r$ obtained for a given F_r when
(a) a "steady state" is reached, *i.e.* fishing in the second and subsequent years is the same, F_{r+1} etc. (b) there is a sudden drop in fishing effort, *i.e.* fishing ceases in the third and subsequent years, $F_{r+2} = F_{r+3} = \ldots = 0$. Values obtained when the fishing effort continues to increase will lie between those for the "steady state" and the curve $k = 1.0$. Dotted line shows the effect of an increase in fishing mortality of 0.1 each year.

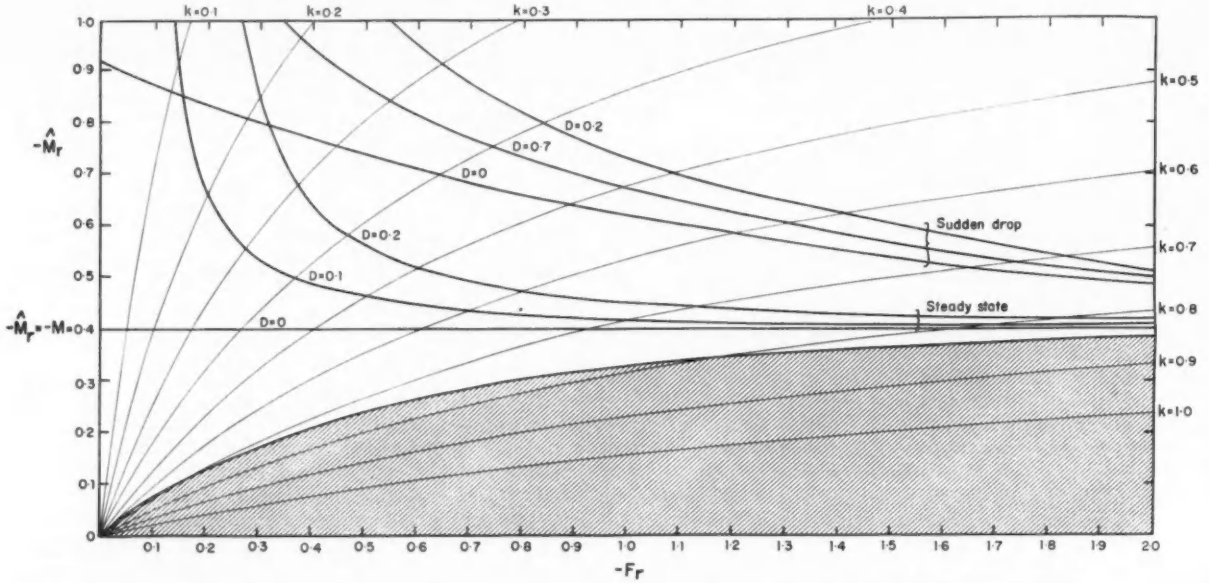

FIG. 4. Estimates of the mortality rate obtained when the true mortality rate, M, is –0.4 and the fishing mortality *decreases* in the second year by different amounts, *i.e.* for $D = 0, 0.1, 0.2$ where $F_{r+1} - F_r = D$.
Heavy lines indicate the values of $\hat{M}_r$ obtained for a given F_r when
(a) a "steady state" is reached, *i.e.* fishing intensity in the second and subsequent years is the same, $F_{r+1} = F_{r+2}$ etc. (b) there is a sudden drop in fishing effort, *i.e.* fishing ceases in the third and subsequent years, $F_{r+2} = F_{r+3} = \ldots = 0$. Values obtained when the initial drop in fishing intensity is followed by an increase will lie between those for the "steady state" and the shaded area.

TABLE I. Effect of changes in fishing effort and natural mortality on the biased estimate of natural mortality, M. The three cases correspond to no change, decrease or increase in fishing effort in the third year. E is the amount of bias, *i.e.* $E = |\hat{M} - M|$. D is the difference between fishing mortality in the first and second years of the virtual population's vulnerability.

	Initial increase in fishing $\lvert F_{r+1} \rvert > \lvert F_r \rvert$, D negative			Initial decrease in fishing $\lvert F_{r+1} \rvert < \lvert F_r \rvert$, D positive		
	$\hat{M}$ is under or overestimate of M	Effect on E of increase in D	Effect on E of increase in M	$\hat{M}$ is under or overestimate of M	Effect on E of increase in D	Effect on E of increase in M
Case (a) "Steady state"	under	increase	increase	over	increase	increase
Case (b) Sudden drop	over	decrease Limit: if D=0	decrease	over	increase	decrease
Case (c) Rapid increase	under	increase Limit: $k=1$ if $D=\infty$	increase	under	increase Limit: if $D=\infty$ $k_r = X_r + e^{F_r+M_r}$	increase

gives a value of E which, although small for high F_r, is greater than the E obtained (for the same absolute value of |D|) when the fishing *increases* between the first and second year before becoming steady; as |D| increases E also increases. An overestimate is also obtained in case (b) when the fishing is reduced in the third year; in the limiting case when fishing ceases the values of E are always greater than those obtained when the curtailment of fishing is preceded by an increase, again as |D| increases E also increases. An underestimate can be obtained in case (c) when the drop in fishing is followed by a period of increased intensity. For such an underestimate E is greatest when $k_r = X_{r+1} + e^{F_{r+1}+M_{r+1}}$ and hence reaches a limiting value when $D = 0$ and $k_r = X_r + e^{F_r+M_r}$. This line is plotted in Figures 2 and 4 and the area below is shaded.

When Figures 2 and 4 are compared the shaded areas show that for the limiting underestimate E is slightly greater when $M = -0.4$. For the steady state, case (a), E increases with M, particularly for low values of F_r. For a sudden drop in fishing, case (b), E increases as M increases throughout the range of values of F_r considered. Thus for the two values of M plotted the possible range of values of $\hat{M}_r$, for any specific positive value of D and any F_r, is not very different but for $M = -0.4$ the line $\hat{M}_r = M$ lies more nearly in the center of the range than it does for $M = -0.2$.

Conclusions from Graphs

It may be seen from Figures 1–4 that E is greater for low values of F_r than for high. This is largely true even if a percentage change is considered, *e.g.* compare E for $|D| = 0.1$ and $F_r = -0.1$ with E for $|D| = 0.2$ and $F_r = -0.2$. Thus for any specific fishing pattern in subsequent years, E is less when the initial fishing intensity is high than when it is low.

Considering the values of E obtained when the fishing in the second and subsequent years is of the same intensity shows that, for a given F_r, a decrease in fishing between years r and $r+1$ of magnitude |D| gives an overestimate slightly more in error than the underestimate that occurs when fishing is increased by D. The "steady state" curves probably give values of $\hat{M}_r$ fairly close to those generally encountered in most fisheries, since small changes in fishing intensity after the second year would give values of k close to $\frac{F_{r+1}}{F_{r+1}+M_{r+1}}$. Similarly comparison of extreme values of $\hat{M}_r$, resulting from violent changes in fishing effort, shows that a very great decrease in fishing in the third year would give an overestimate of M with a value of E very much greater than that which would occur with a violent increase in fishing and consequent underestimate of M.

EFFECT OF LINEAR REGRESSION ON ESTIMATES OF M

If, as suggested on page 77, c is assumed to be unity so that $f_r = F_r$, then M^* is the arithmetic mean of $\hat{M}_r$ and it seems at first sight, from consideration of the range of $\hat{M}_r$ and the probable values of E for a given F_r, that M^* is an overestimate of M. In practice however c is not unity and it is represented by the

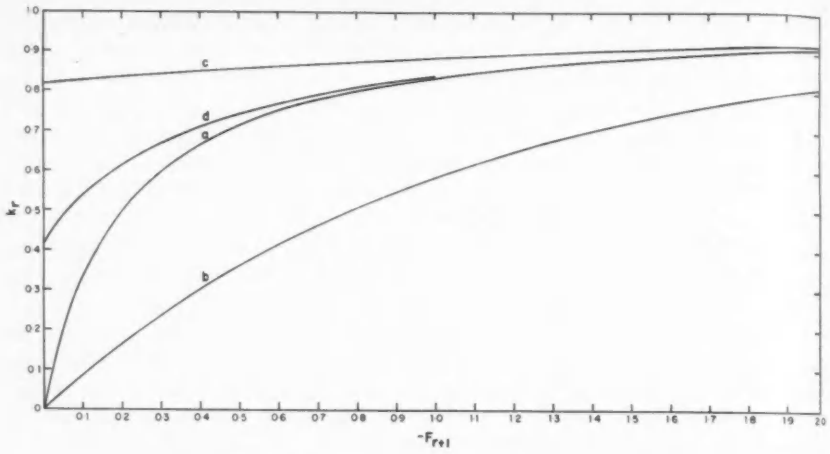

FIG. 5. Relationship between k_r and F_{r+1} when $M = -0.2$ and

(a) $k_r = \dfrac{F_{r+1}}{F_{r+1}+M_{r+1}}$ *i.e.* fishing remains in a "steady state" after the second year.

(b) $k_r = X_{r+1}$ *i.e.* fishing ceases in the third year and is not resumed.

(c) $k_r = X_{r+1}+e^{F_{r+1}+M_{r+1}}$ *i.e.* maximum fishing in the third year such that all remaining fish are caught instantly.

(d) $k_r = X_{r+1}+e^{F_{r+1}+M_{r+1}}X_{r+2}+e^{F_{r+1}+M_{r+1}+F_{r+2}+M_{r+2}}X_{r+3}+$ etc. where F increases by 0.1 each year.

slope of the linear regression of $\log(z_r)$ and f_r, while M* is the intercept on the $\hat{M}$-axis. It is therefore necessary to consider the distribution of $\log(z)$ or in other words to consider the bias E in each $\hat{M}_r$. During a period of small fluctuations in fishing effort with no marked trend, the highest values of f_r (and hence F_r) would be followed by lower values, *i.e.* for high F_r there would be a decrease in the second year and, vice-versa, for low F_r there would be an increase in the second year. Thus $\hat{M}_r$ would be an overestimate of M in the upper range of F_r values and an underestimate in the lower range. Hence $\log(z_r)$ would be an overestimate of $cf_r + M$ in the upper ranges of f_r and vice-versa. This would give a value of c that was too large and the intercept value of M* would be an underestimate of M. Consideration of the distribution of E with respect to F_r also indicates that even if it were possible to determine c independently so that the mean of the $\hat{M}_r$'s could be computed it is still likely that M* would be an underestimate of M since for more moderate changes in F_r the error E is larger for small $|F_r|$ than for large; this difference with F_r outweighs the tendency of overestimates to be more in error than underestimates for a specific F_r.

During the period when there is a trend in the fishing effort all the values of $\hat{M}_r$ will lie close to the "steady state" curves. For a gradual increase in fishing

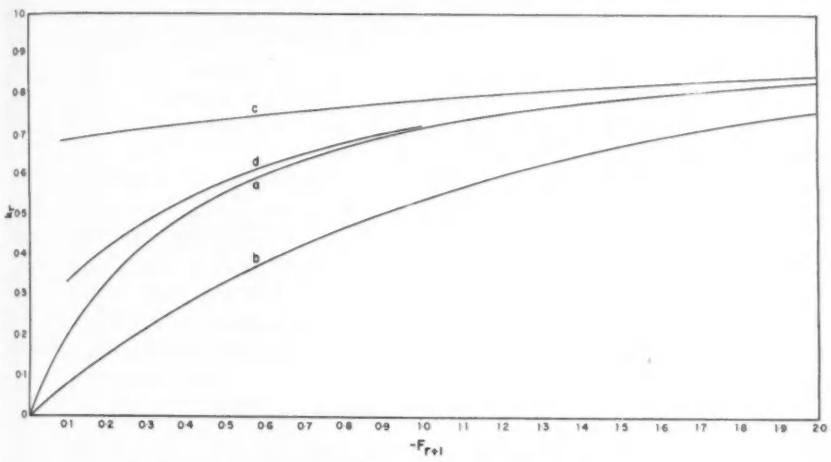

FIG. 6. Relationship between k_r and F_{r+1} when $M = -0.4$ and

(a) $k_r = \dfrac{F_{r+1}}{F_{r+1}+M_{r+1}}$ *i.e.* fishing remains in a "steady state" after the second year.

(b) $k_r = X_{r+1}$ *i.e.* fishing ceases in the third year and is not resumed.

(c) $k_r = X_{r+1}+e^{F_{r+1}+M_{r+1}}$ *i.e.* maximum fishing in the third year such that all remaining fish are caught instantly.

(d) $k_r = X_{r+1}+e^{F_{r+1}+M_{r+1}}X_{r+2}+e^{F_{r+1}+M_{r+1}+F_{r+2}+M_{r+2}}X_{r+3}+$ etc. where F increases by 0.1 each year.

effort all the values of $\hat{M}_r$ will be underestimates of M, the intercept value M* will also be an underestimate and, since E will be larger for low F_r than for high, the value of c will be overestimated. During a period when the fishing effort is declining each $\hat{M}_r$ will be an overestimate of M, so will M*, and c will be underestimated. Thus during a period of gradual decrease of fishing effort followed by gradual increase it is possible to obtain a value of M* close to the true value of M, and also an accurate estimate of c, as was done by Fry in the example given on page 73. It is also interesting to plot his values: it is observed that his greatest deviations from the linear regression occurred during the period when the trend in fishing was changing; it has been shown in some detail in the foregoing that the greatest bias occurs when fishing is fluctuating.

CONCLUSIONS

It is concluded that since each individual estimate of total mortality is biased, the virtual population method will generally give an underestimate of the natural mortality and an overestimate of the coefficient of fishing mortality. The only exception to this seems to be when there is a gradual decline in fishing effort when,

for the period of years for which this trend persists, the errors in both mortality rates will be in the opposite direction. The bias in individual estimates will be more pronounced when there are large fluctuations in fishing effort, particularly when the fishing mortality is low relative to the natural mortality. The bias of individual estimates is also likely to be greater for a higher natural mortality rate and hence the final estimates may be more in error.

Auxiliary possible sources of error can be evaluated relatively from the Figures. Changes in the vulnerability of the stock would mean that f_r was not directly proportional to the fishing mortality, and would produce the same effect as an error in f_r, *i.e.* the bias E would be the same as if M_r were plotted against the wrong F_r in the year r, and D would be misjudged in the preceding year. In the upper ranges of F_r this would not increase the bias too much. It may also be noted that the method is entirely independent of the size of the recruitment, R, to each year-class.

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Three-year-old Pink Salmon[1]

BY RAYMOND E. ANAS

U.S. Fish and Wildlife Service
Seattle, Washington

ABSTRACT

A specimen of *Oncorhynchus gorbuscha* 552 mm in fork length, taken in the Skeena River fishery in 1956, had scales indicating two years of growth completed and substantial 3rd-year growth.

PINK SALMON *(Oncorhynchus gorbuscha)* mature in two years. This characteristic is so dominant that the author could find no reference in the literature to a 3-year-old pink salmon from a North American stream. Occurrence of pink salmon older than two years has been reported in Asia, but these age interpretations are still controversial (Semko, 1954).

All pink salmon aged in the Seattle laboratory of the U.S. Fish and Wildlife Service have been 2-year-olds, with one exception. A 3-year-old pink salmon, a female, was found in samples from the Skeena River, British Columbia, collected for the International North Pacific Fisheries Commission in 1956. Figure 1 is a photograph of a scale from the 3-year-old showing the two annuli; Fig. 2 is a photograph of a typical 2-year-old pink salmon scale. The 3-year-old was identified visually in the field and in the laboratory as a pink salmon; the small scales of this species are characteristic and useful for identification. Also, the gill raker count of 11 dorsal and 17 ventral rakers identifies the 3-year-old as a pink salmon (Schultz, 1936; Clemens and Wilby, 1946).

The 3-year-old pink was 552 mm in fork length, while the 61 2-year-old fish with which it was taken ranged from 422 to 604 mm; its mid-eye to hypural plate length was 470 mm, as compared with 352–501 mm for the 2-year-olds. The weight of the 3-year-old fish was 2.42 kg, as compared with 1.03–3.43 kg for the 2-year-olds. Comparisons of scale measurements are shown in Table I.

TABLE I. Number of circuli and scale measurements (in mm × 42.5) of the 3-year-old pink salmon and of the sample of 61 2-year-old pinks in which it occurred. For the latter the mean, range and 99% confidence limits are shown.

	Number of circuli			Length in mm × 42.5		
	Mean	Range	99% limits	Mean	Range	99% limits
Focus to first annulus						
Age II	26	22–32	20–31	38	30–46	29–48
Age III	28	...	...	47	...	...
First annulus to edge of scale						
Age II	17	13–21	12–21	33	21–45	20–46
Age III	33	...	...	50	...	...
Total scale						
Age II	42	37–48	36–49	71	59–91	54–88
Age III	61	...	...	97	...	...
First annulus to second annulus						
Age III	26	...	...	38	...	...

[1]Received for publication May 28, 1958.

These data substantiate the visual determination that the pink salmon was 3 years old. The only scale data that are comparable between the sample and the 3-year-old are in the zone from the focus to the first annulus. All other scale data for the 3-year-old are beyond the 99% confidence limits of the sample range of values that would be expected in a *t*-distribution.

ACKNOWLEDGMENT

K. H. Mosher, U.S. Fish and Wildlife Service, corrected the manuscript and photographed the scales in Figures 1 and 2.

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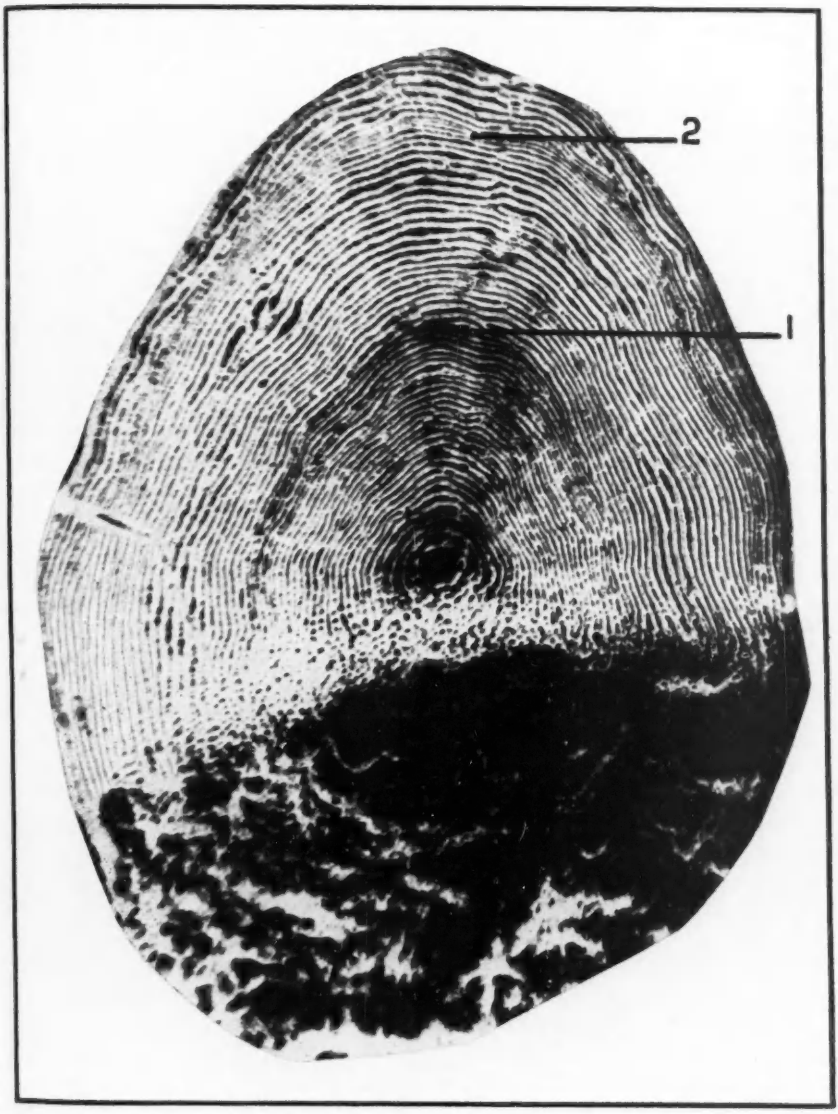

FIG. 1. Photograph of a scale of the 3-year-old pink salmon, showing the two annuli.

FIG. 2. Photograph of a typical 2-year-old pink salmon scale.

Muscular Fatigue and Mortality in Troll-Caught Chinook Salmon (*Oncorhynchus tshawytscha*)[1, 2, 3]

By Robert R. Parker[4] and Edgar C. Black[5]

ABSTRACT

Blood samples were analyzed for lactic acid from 66 troll caught chinook salmon after from zero to 10¾ hours of rest. Fish were held in a live box aboard a trolling vessel. During the course of the experiment 22 individuals died. Analysis of these data indicate a general response of blood lactic acid comparable with results of other experiments. The typical response is a gradual increase of blood lactic acid to high levels in the third and fourth hours, followed by a general decline. Death is strongly associated with high blood lactic acid. The level of lactic acid response is not significantly increased with more than 10 minutes of vigorous exercise nor is it significantly affected by the size of the fish within the ranges sampled in this experiment. Mortality rate is estimated at 71% with 95 percent binomial confidence limits of 40% and 86%.

INTRODUCTION

That fish die following severe exertion was first shown by von Buddenbrock (1938), working with cod (*Gadus morrhua*) and the dab (*Platessa limanda*). Huntsman (1938) further discussed the problem in a review article and concluded that struggling of fish in captivity led to death. Secondat and Diaz (1942) have also shown that tench (*Tinca tinca*) may die following severe exercise. In 1956, death of Pacific salmon following severe muscular exercise was demonstrated by Bates and Vinsonhaler (1957) for smolt chinook salmon (*O. tshawytscha*), and by Black (1957c) working on two-year-old sockeye salmon (*O. nerka*) acclimated and exercised in sea water. Paulik and DeLacy (1958) have reported a reduction in survival time of mature sockeye salmon after strenuous activity in fresh water. Bates and Vinsonhaler also noted death following severe muscular exercise of immature striped bass (*Roccus saxatalis*) and shad (*Alosa sapidissima*). Presumably death in some way resulted from muscular exercise.

Von Buddenbrock (1938) noted the presence of lactic acid in the blood and attributed death to a suppression of oxygen transport ability, *i.e.* to asphyxiation. Secondat and Diaz (1942) and Black (1957a, b, c) demonstrated the presence of high concentrations of lactic acid in the blood of fish following severe exercise. These latter authors standardized exercise as maximum swimming effort sustained for 15 minutes. They also demonstrated that blood lactic acid levels continue to rise after the exercise period, reaching a maximum during the first 1 or 2 hours of post-exercise rest, and then may remain at a high level for a subsequent 2 to 6 hours.

[1]Received for publication February 19, 1958.
[2]Contribution from the Alaska Department of Fish and Game, Juneau, Alaska, and from the Department of Physiology and the Institute of Fisheries, University of British Columbia, Vancouver, Canada.
[3]This project was supported in part by a grant-in-aid from the National Research Council of Canada, and by the British Columbia Electric Company, Vancouver, Canada.
[4]Senior Biologist, Alaska Department of Fish and Game, Juneau, Alaska; Graduate Student, Institute of Fisheries, University of British Columbia, Vancouver, Canada.
[5]Associate Professor, Department of Physiology, University of British Columbia, Vancouver, Canada.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

At the present time the precise cause of death in fishes following severe muscular activity is not known; Black (1958) presents a review of the available evidence. Whatever the mechanism involved, the effects of fatigue upon survival are an important consideration in studies involving tagging or in management of a stock of fish by a size restriction. Increase of lactic acid concentration in the blood appears to be directly correlated with, if not a causative agent of, death after severe muscular exertion. Death due to fatigue is delayed (Black 1957c; Paulik and DeLacy, 1958) and the survival of an individual fish cannot be predicted from external appearances at the time of catching. In view of these observations the present experiment was designed to examine the delayed effects of commercial fishing by troll gear on the survival of released chinook salmon. Levels of lactic acid in the blood appear to provide a good indicator of the state of well-being of the fish following exercise; thus, it was chosen as the dependent variable. The authors also wished to determine to what extent a fish can be exercised without mortality as well as the differences in effects of exercise on different sized fish. Specimens obtained aboard a commercial troller offer such opportunity, as both time spent on the gear and size of individual fish vary considerably in a normal fishing operation.

The experiment was conducted during the latter half of August, 1957, on fishing grounds at approximately 58° 40′ N., 138° 15′ W., about 15 miles off Cape Fairweather, Alaska.

METHODS AND MATERIALS

Chinook salmon were obtained aboard a commercial trolling vessel. All elements of catching, *i.e.* place, depth, and gear used were left to the discretion of the Captain in order to test survival of fish caught under strictly commercial fishing conditions. Six lines were fished, each line bearing from 8 to 12 lures. Lures used were a standard brand of commercial spoons with No. 7 or No. 8 hooks.

Every effort was made to record the time each fish was on the line; this was complicated in several instances by fish biting other lures on the same line, necessitating a guess as to which was the original fish. The time statistic is referred to as the "exercise time". Fish used for the experiment were landed and placed in a live box, then the lure was removed.

Rested, unexercised fish were impossible to obtain for a standard base line. Lactic acid values for fish immediately after exercise were obtained by sampling 6 individuals as soon as they were landed. Two of these were bleeding from gill injuries. All fish held were in what appeared to be good condition and bore no visible signs of damage to the gills, eyes or other vital organs. In other words, fish tested were selected for maximum survival as judged from external appearances, and would have been considered fit for tagging.

The live box contained approximately 750 litres (200 gallons) of sea water and was lined with polyethylene sheet. Horizontal dimensions were approximately 3.0 by 5.5 feet and water depth was maintained at approximately 1.5 feet. Fresh sea water was continually supplied by means of a mechanical pump at a rate of approximately 75 litres (20 gallons) per minute.

Individual fish were coded by using a stainless cattle tag bearing a serial number, applied dorsally to the caudal fin. Fish were allowed to rest in the live box for predetermined amounts of time, the objective being to obtain samples over a 10 hour range. In no case were more than 4 fish held in the live box at one time.

Surface water supplied to the live box was between 14° and 15°C. Fishing took place over depths ranging from 70 to 80 metres (35–40 fathoms) and the majority of fish took lures at or below 30 metres (15 fathoms). Several bathythermograph casts were made during the course of the experiment. Fish utilized generally came from water of a temperature between 7° and 10°C. The possible significance of this change in temperature to blood lactic acid levels is not known. In general, the rate of metabolism would be increased by a change of this magnitude but diffusion rates into and out of the blood would also increase. The interaction of these changes cannot, at present, be predicted.

The fish were disturbed as little as possible during the resting period; however, motion of the vessel and vibration of the propulsion engine could not be controlled. When fish were first placed in the live box, they generally appeared lively and would explore the tank. This activity lasted but a few minutes and was followed by quiescence. Often fish would fail to maintain equilibrium and float passively upside down, either at the surface or on the bottom. This condition usually appeared after one half hour of rest, although some individuals would lie over when first introduced into the tank. Some fish righted themselves after various amounts of rest, others failed entirely to do so. Similar behaviour was observed by the second author in studies with yearling rainbow trout and two-year-old sockeye salmon.

Judging from the appearance of the gonads, and from results of previous studies in the same area (Parker and Kirkness, 1956), fish used in the present experiment were not in their ultimate year. All fish taken were feeding, many had gorged stomachs. Thus the present experiment contrasts with that of Paulik and DeLacy (1958) who used mature non-feeding fish in fresh water.

At the end of a selected time period, if the particular fish did not die, it was stunned by a blow on the head and blood was immediately drawn from the heart. In case the fish died prematurely, a blood sample was taken before clotting and before *rigor mortis*. At the time of stunning the "apparent condition" of the fish was subjectively evaluated and recorded.

After withdrawing the blood sample the fish was again examined for external injury, and fork length to the nearest half inch was recorded.

One millilitre of blood was drawn from the heart into a 2-ml Luer syringe coated with mineral oil and rinsed with heparin solution. The sample was immediately expelled into a polyethylene bottle containing 9 ml of 10% trichloracetic acid. This mixture was filtered within the hour and the filtrate, collected in a polyethylene bottle, placed on ice in the fish hold. The samples were taken to the Department of Physiology of the University of British Columbia in September and the analyses for lactic acid by the method of Barker and Summerson (Hawk, Oser, and Summerson, 1949) were carried out by the second author in October.

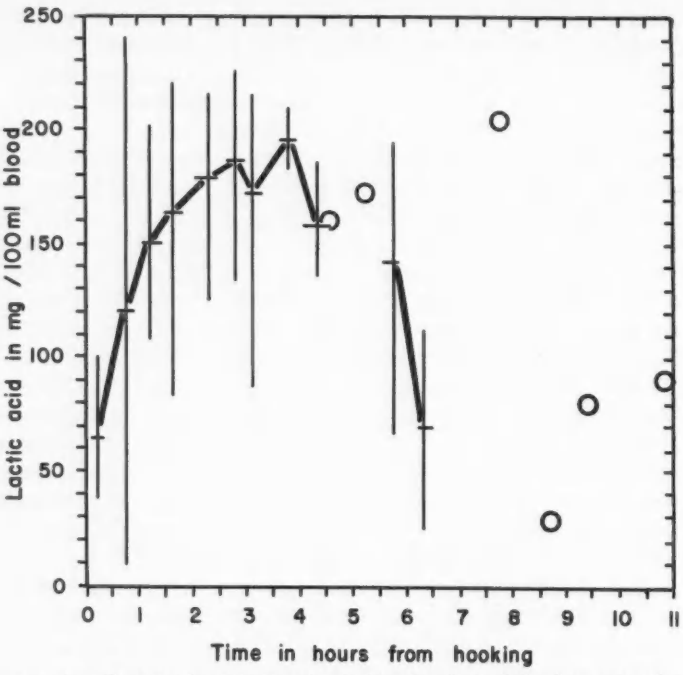

FIG. 1. Blood levels of lactic acid of chinook salmon following capture by trolling. The averages are intercepted horizontally by lines representing the time range of the group, and vertically by a line representing the range in lactic acid. Single observations are notated by a circle.

The values of lactic acid are expressed as milligrams of lactic acid per 100 millilitres of whole blood (mg%).

RESULTS

A total of 66 samples was obtained, which included fish given from zero up to 10¾ hours of rest. Within the total sample, the exercise time varied from 3 to 30 minutes, and the fork length of individuals varied from 38 to 85 cm (15.0 to 33.5 inches). These data are presented in the appendix. The total sample was first grouped according to half-hour time periods, zero time being taken from the start of exercise period (at hooking). Within these groups mean lactic acid level in mg% and mean time were computed. These data, together with the within-group ranges are graphically presented in Fig. 1 and recorded in Table I.

A large variation is noted in these samples and it was desirable to test for significance not only of curvilinearity but also the effects of size and exercise differences in contributing to systematic error. From an inspection of Fig. 1, it appears that the means of observations (exclusive of the four highest time periods) might best be described by a parabola[6]. The four individuals representing the four

[6]We do not consider a parabola in itself to have any significance, *i.e.* the choice was one of statistical convenience.

TABLE I. Summary of data for total time of exercise and holding in live box, and blood levels of lactic acid in Chinook salmon.

Number of fish	Time period *hours*	Time, *minutes*		Blood lactic acid, *mg %*	
		Range	Mean	Range	Mean
6	0 – 0.5	9–20	14	38.8–101.0	64.6
6	0.5–1.0	35–55	46	9.6–240.0	120.1
8	1.0–1.5	65–88	74	108.0–202.0	150.1
10	1.5–2.0	91–112	98	83.2–220.0	163.1
7	2.0–2.5	126–150	138	125.0–216.0	177.9
5	2.5–3.0	160–180	169	134.0–226.0	185.6
5	3.0–3.5	183–200	190	87.3–216.0	171.5
2	3.5–4.0	220–235	228	182.0–209.0	195.5
3	4.0–4.5	245–274	261	136.0–186.0	157.7
1	4.5–5.0	...	275	...	160.0
1	5.0–5.5	...	315	...	172.0
6	5.5–6.0	333–354	346	66.6–195.0	141.8
2	6.0–6.5	375–382	378	26.0–113.0	69.5
0	6.5–7.0	...	...	...	...
0	7.0–7.5	...	...	...	...
1	7.5–8.0	...	463	...	204.0
0	8.0–8.5	...	...	...	...
1	8.5–9.0	...	520	...	29.0
1	9.0–9.5	...	562	...	80.5
0	9.5–10.0	...	...	...	...
0	10.0–10.5	...	...	...	...
1	10.5–11.0	...	645	...	89.7

highest time periods were excluded from the data for these calculations, *i.e.* only the time period from 0 to 6.5 is considered. For statistical convenience the data were coded into groups (Table II).

For covariance analysis of size, fork lengths (L, in inches) were first converted into estimated weights (W, in pounds dressed) by the empirical formula of Parker and Kirkness (1956):

$$W = \frac{L^{3.15}}{354}$$

This conversion was thought necessary as weight represents mass of fish producing lactic acid better than a linear dimension. Individuals were classified into three weight groups: (1) 2700 grams (6.0 pounds) or less; (2) 2700 to 5500 grams (6.1–12.0 pounds); (3) more than 5500 grams (>12.1 pounds). Individuals were also classified into two groups according to recorded exercise time: (1) 10 minutes and less, (2) 11 minutes and more. A further classification was desirable to test for any correlation between high lactic acid levels and death. This classification is arbitrary in that no knowledge is available as to the probable fate of individuals killed for blood samples. The entire classification system is presented in Table II.

A second degree polynomial was fitted to the total sample by the method of multiple regression (Snedecor, 1950), yielding, when converted to ordinary units, the equation:

$$\hat{Y} = 6.41 + 1.848X - 0.067X^2$$

The standard error of estimate, $s_{y.12}$, is 4.365 cells of Y or 43.65 mg%. The calculated curve together with the coded cells of empirical data and their frequency are presented in Fig. 2. A test for significance of departure from linear regression

TABLE II. Coding and grouping of time held after hooking (X) and blood levels of lactic acid (Y).
X = intervals of ¼ hour from time of hooking to time of death.
Y = intervals of 10 mg % lactic acid concentration.

	Exercise groups							
	Less than 10 minutes				More than 10 minutes			
	Alive		Dead		Alive		Dead	
N	X	Y	X	Y	X	Y	X	Y
Fish less than 2700 grams (6 lb)								
1	1	7	5	21	3	1	7	21
2	3	9	6	16	3	24	9	22
3	4	10			8	22	9	16
4	5	13			21	18	11	23
5	7	12			23	14	12	20
6	7	9					13	22
7	8	16					15	21
8	12	14					17	19
9	13	9						
10	19	16						
Fish of 2700 to 5500 grams (6–12 lb)								
1	1	4	5	11	4	15	7	17
2	1	4	9	18	13	20	10	17
3	4	15	16	19	23	7	10	22
4	5	14			25	12	13	22
5	6	19					18	14
6	7	20						
7	7	16						
8	7	16						
9	18	16						
10	23	20						
11	24	20						
12	24	9						
13	26	3						
Fish of more than 5500 grams (12 lb)								
1	5	13	9	19	1	9		
2	6	18	11	18	1	11		
3	7	18			2	6		
4	10	13			14	15		
5	12	17						
6	23	18						

gave a significant "F" value of 34.0, d.f. = 1, 59. The correlation between $\hat{Y}$ and Y (estimated mean regression and observed values) is R = 0.617, where significance at $P_{.01}$, with three variables is at R = 0.410.

Analysis of covariance was used to test for systematic error due to both size and exercise time. Size proved non-significant, "F" = 0.3, d.f. 2, 48; exercise time was also non-significant, "F" = 2.5, d.f. 1, 48 ($P_{.05} = 4.04$). A significant difference was obtained between live fish and fish which died, "F" = 13.8, d.f. 1, 48. A regression line was calculated for the dead group only obtaining the equation:

$$\hat{Y} = 2.580 + 2.863X - 0.111X^2, \quad s_{y.12} = 2.681.$$

This curve is plotted as a dashed line in Fig. 2 and is seen to lie above the regression line of the total sample.

As previously noted, 22 fish died during the course of the experiments (Table II). This number does not, however, represent any estimate of mortality *per se* in

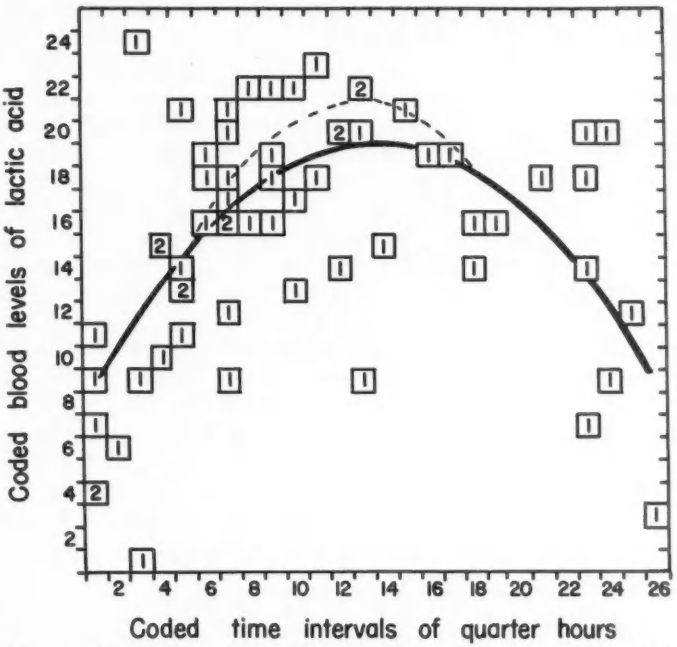

FIG. 2. Calculated parabola (solid line) from coded blood levels of lactic acid in quarter-hour time periods from hooking. See text and Table II. Solid line, all data; dashed line, dead only.

that individuals were also killed, and no objective measurements of their chances for survival in lieu of sampling are available. This difficulty may be overcome by making an assumption that at any time period, fish killed were a random sample of fish surviving to that period. The validity of this assumption appears to depend upon unbiased determination of which fish to kill, a condition thought to be satisfied in that the period of post-exercise rest for each individual was determined prior to catching. Calculation of point estimates and 95 percent binomial confidence limits are presented in Table III. Instantaneous mortality rates[7] (i) for each time period were computed from the number dying and the number killed during a period from the number surviving the preceding period. Variance of (i), s_i^2, is estimated by $s_i^2 = s_p^2/p^2$ (Deming, 1943, p. 45), where p is the estimated probability of survival for each period. Instantaneous mortality rates and their variances were then accumulated for each successive period. Confidence limits of accumulated i (I) for each successive time period, denoted as $\underline{I}$, $\bar{I}$, were estimated from the binomial approximation. At the 95 percent level, $\underline{I}_t, \bar{I}_t = I_t \pm 1.96\, s_{I_t}$. Absolute mortality rates (m) were calculated by the relationship $m = 1 - e^{-I}$ yielding an estimate of observed mortality rate (0.71) and an estimate of the magnitude of

[7]Instantaneous mortality rate (i) = $\log_e N_1 - \log_e N_2$ where N_1 denotes corrected total of fish surviving period 1 and N_2 denotes fish surviving period 2. $N_2 - N_1$ = fish dying.

TABLE III. Schedule of calculations of mortality estimates (see text).

Time period (t) (*hours*)	Survivors			Deaths	i_t	Cumulative mortality rates					
						Instantaneous			Total to end of period		
	Held	Killed	Diff.	(d)		I_t	I_t	$\bar{I}_t$	$\underline{m}_t$	m_t	$\bar{m}_t$
						[c]	[b]	[c]		[d]	
0–1	66	12	54	0	0	0	0	0	0	0	0
1–2	54	13	41	5	0.1301[a]	0.0160	0.1301	0.2442	0.016	0.122	0.216
2–3	36	3	33	9	0.3185	0.2318	0.4486	0.6654	0.207	0.362	0.486
3–4	24	3	21	4	0.2113	0.3598	0.6599	0.9600	0.302	0.483	0.617
4–5	17	2	15	2	0.1431	0.4433	0.8030	1.1627	0.358	0.552	0.687
5–6	13	6	7	1	0.1542	0.4872	0.9572	1.4272	0.385	0.616	0.760
6–7	6	2	4	0	0	0.4872	0.9572	1.4272	0.385	0.616	0.760
7–8	4	0	4	1	0.2877	0.5095	1.2449	1.9803	0.400	0.712	0.862

[a] Ln 41 − Ln 36 = 0.1301 = i = instantaneous mortality rate for the second time period.

[b] $I_t = \sum_{t=1}^{n} i_t$, where I = the cumulative instantaneous rates to include any time period n.

[c] $s^2_{I_{t=2}} = \frac{sp^2}{p^2} = \frac{5}{41 \times 36}$; $s^2_{I_{t=n}} = \sum_{t=1}^{n} s^2_{i_t}$; $\underline{I}_t, \bar{I}_t = I_t \pm 1.96\, s_{I_t}$, which estimates the 95% binomial confidence interval.

[d] $m_t = 1 - e^{-I_t}$ = total mortality rate including time period t.

expected mortality rate (0.40 to 0.86) for fish held under similar conditions for 8 hours.

The results are summarized as follows:

1. Blood levels of lactic acid rise from a mean level of less than 60 mg% to an order of magnitude of 180 mg% during the first three hours from hooking. These high levels persisted through the fourth hour and then declined toward normal.
2. The magnitude of this general response was not significantly correlated with the size of the individual.
3. The general response was precipitated by less than 10 minutes of struggling and further time on the troll gear did not significantly alter the degree of response.
4. Death of individuals was significantly associated with high blood levels of lactic acid.
5. Mortality of the experimental lot of fish was estimated to be 71%, and the 95 percent binomial confidence limits indicate an expected mortality between 40% and 86% for uninjured troll-caught chinook salmon subjected to similar treatment.

The large variation in degree of lactic acid response is thought to reflect individual variation in respect to muscle glycogen level due to either previous environmental experience or genotypic differences, as well as a variable degree of exertion on the troll gear.

DISCUSSION AND CONCLUSIONS

Black and Barrett (1957), working with trout (*Salmo clarki* and *S. gairdneri*), demonstrated that blood lactic acid increases as a result of handling. However, these responses were much lower (highest response approximately 55 mg%) than levels noted by Black (1957a) for 15 minutes of forced exercise (*S. gairdneri*, above 140 mg%).

Chinook held in the live box were generally quiescent; many were completely unresponsive to normal stimuli as evidenced by loss of equilibrium. Further, a direct comparison with Black's (1957c) experimental sockeye shows a similar sequence of events although the details contrast. These experiments are compared in Table IV.

TABLE IV. Comparison of mortality data for chinook (this paper) and sockeye (Black, 1957c) during recovery period following exercise.

	Chinook	Sockeye
Water temperature	15°C	20°C
Number exercised	66	19
Number dying	22	5
Weight, average	3640 g (8.0 lb)	198 g (7 ounces)
Time to death, average	169 minutes	90 minutes
Lactic acid just after exercise, average	64.6 mg %	103 mg %
Lactic acid at death, average	189 mg %	240 mg %

Since lactic acid arises from hydrolysis of muscle glycogen in the absence of or at low concentrations of oxygen, there can be little doubt that the observed changes are due to exertion on the hook and not to conditions of holding fish on

board the vessel. Further, the degree of lactic acid response is shown to be significantly correlated with the occurrence of death. The possibility of psychosis leading to death from close confinement exists; however, death also occurred in Black's (1957c) tame (hatchery raised) sockeye following forced exercise.

An experiment, similar in several respects to the present one, is reported by Milne and Ball (1956). These authors held troll-caught coho salmon (*O. kisutch*) aboard a research vessel from 1 to 6 hours and then transferred them to a live pond. Of 55 individuals considered suitable for tagging, 11 died while in the live tank aboard the vessel. This fraction, converted to percent mortality, is 20%. The 0.95 binomial confidence interval is 10% to 30%. This confidence interval may be compared with that for chinook held 4 hours (Table III), 30.2% to 61.7%. Thus Milne and Ball's data yield an estimate of mortality that is clearly lower than that of the present experiment. This discrepancy may be due to the species used or to differences in experimental conditions as well as the variable holding time of the coho.

The present study has not demonstrated that the death of the chinook salmon was due exclusively to the severity of forced exertion. Other factors may have contributed to, or possibly have been primary causes of, death, *e.g.* unrecognized internal injury, psychosis from close confinement, handling, abrasion of the mucous coat. Nevertheless, the authors are of the opinion that the principal factor causing death was severe muscular exercise. The average degree of muscular work done by chinook while on troll gear is much more than normally occurs while the fish is free in its environment, hence no biological adaptation for work of this intensity is present. Fatigue to this degree is often fatal. Thus fatigue is an important consideration in planning a tagging experiment and must be considered in any evaluation of benefits expected to accrue from a size regulation.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge Captain Ingvold Ask for the part he played in the present study. In addition to the facilities provided free of charge aboard his vessel *Scenic*, Captain Ask took a personal interest in the progress of the field work and assisted in numerous ways. Dr P. A. Larkin, Director of the Institute of Fisheries, Dr S. W. Nash, Department of Mathematics, University of British Columbia and Dr W. E. Ricker of the Fisheries Research Board of Canada, contributed to the statistical analysis. Dr C. C. Lindsey, Institute of Fisheries, University of British Columbia, critically read the manuscript. To the Alaska Department of Fish and Game, through Director C. L. Anderson, to the National Research Council of Canada and to the British Columbia Electric Company the authors are indebted for funds, laboratory supplies and facilities made available.

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APPENDIX. Summary of raw data pertinent to fatigue-survival study, chinook salmon.

Sample number	Time on gear	Time in live box	Apparent condition when landed	Apparent condition when sampled	Fork length	Estimated weight	Lactic acid	Serious injuries noted
	min.	*hours & minutes*			*inches*	*pounds*	*mg%*	
2	5	0–4	lively	—	28.0	10.2	38.8	none
3	3	3–0	lively	weak	16.5	1.9	87.3	none
7	5	1–30	lively	weak	21.0	4.1	117.	none
8	10	1–30	lively	weak	23.5	5.9	83.2	none
10	10	0–5	weak	—	28.5	10.9	38.8	bled badly
11	10	1–0	lively	dead	23.5	5.9	202.	none
12	10	1–0	lively	dead	24.5	6.7	108.	none
13	10	1–0	tired	weak	19.5	3.3	122.	none
14	8	2–40	lively	weak	32.5	16.4	194.	none
15	15	0–0	lively	—	30.0	12.7	88.4	none
16	15	0–0	lively	—	33.0	17.2	101.	none
17	9	0–45	lively	weak	21.5	4.4	93.7	none
18	7	0–45	lively	weak	24.0	6.3	145.	none
19	7	2–20	lively	weak	31.5	14.8	125.	none
20	15	0–40	lively	weak	26.0	8.1	149.	hook near eye
21	6	1–30	lively	weak	24.0	6.7	198.	hook near eye
22	7	1–25	lively	weak	26.5	8.6	160.	none
23	10	4–25	lively	lively	23.5	5.9	160.	none
24	15	3–0	lively	dead	25.0	7.2	216.	none
25	8	1–10	lively	weak	24.5	6.7	185.	none
26	6	2–0	lively	dead	29.5	12.1	190.	none
27	15	0–30	lively	weak	22.0	4.8	240.	bled
28	15	0–20	lively	weak	19.0	3.0	9.6	none
29	22	9–0	lively	lively	25.0	80.5	7.2	none
31	8	1–20	weak	very weak	30.0	12.7	174.	none

APPENDIX—*continued*

Sample number	Time on gear	Time in live box	Apparent condition when landed	Apparent condition when sampled	Fork length	Estimated weight	Lactic acid	Serious injuries noted
	min.	*hours & minutes*			*inches*	*pounds*	*mg%*	
32	6	1–25	lively	weak	26.0	8.1	160.	none
33	7	1–25	lively	weak	30.5	13.4	172.	none
34	10	0–55	lively	very weak	32.5	16.4	121.	none
35	20	3–45	lively	dead	22.0	4.8	186.	none
36	15	2–55	lively	very weak	25.5	7.7	193.	none
37	10	1–0	lively	very weak	24.5	6.7	137.	none
38	15	2–0	lively	dead	22.5	5.1	216.	none
39	10	0–25	weak	very weak	23.0	5.5	83.2	none
40	7	1–45	weak	very weak	21.0	4.1	157.	none
41	10	3–45	lively	dead	24.0	6.3	182.	none
42	15	2–10	weak	dead	25.0	7.2	168.	none
43	9	0–0	lively	—	23.5	5.9	63.7	gill damaged
44	15	1–10	lively	dead	25.0	5.9	152.	none
45	10	2–0	lively	dead	25.0	7.2	177.	none
46	8	7–35	lively	dead	28.5	10.9	204.	none
47	20	0–0	lively	—	32.0	15.6	56.7	none
48	14	4–20	lively	dead	27.0	9.1	136.	none
50	30	1–10	weak	dead	29.0	11.4	163.	none
51	15	1–55	weak	dead	23.0	5.5	153.	none
52	11	1–40	lively	dead	23.0	5.5	201.	hook near eye
53	20	2–10	lively	dead	25.0	7.2	216.	none
54	15	2–50	lively	dead	20.5	3.8	212.	none
55	15	5–25	lively	lively	21.0	4.4	134.	none
56	20	10–25	lively	lively	23.0	5.5	89.7	gill damage
57	15	3–25	lively	dead	23.5	5.9	209.	none
58	15	3–5	weak	weak	33.5	18.0	149.	none
59	10	2–45	lively	lively	15.0	1.4	134.	none
60	15	2–35	weak	dead	23.5	5.9	200.	none
62	7	6–15	lively	lively	26.5	8.6	26.0	none
63	8	5–25	lively	dead	24.5	6.7	195.	none
64	10	5–50	weak	weak	25.0	7.2	192.	none
65	15	5–0	weak	very weak	22.5	5.1	172.	none
66	15	6–0	lively	lively	26.0	8.1	113.	none
67	15	2–25	lively	dead	23.0	5.5	226.	none
68	15	1–35	lively	weak	22.0	4.8	220.	gill damage
69	10	5–35	weak	weak	31.0	14.1	177.	none
70	10	4–15	weak	weak	29.0	11.4	151.	none
71	10	8–30	weak	lively	28.5	10.9	29.0	hook near eye
72	4	5–50	weak	lively	26.0	8.1	86.3	none
73	10	2–35	weak	dead	30.0	12.7	174.	gill damage
74	13	5–30	weak	lively	26.0	8.1	66.6	none

A Seven-year Study of the Fishery for Lake Whitefish, *Coregonus clupeaformis*, on Lake Winnipeg[1]

By L. C. Hewson[2]

Fisheries Research Board of Canada,
Biological Station, London, Ont.

ABSTRACT

A representative section of the Lake Winnipeg whitefish fishery was studied during seven summers. The average size of whitefish in samples numbering from 100 to 3,500 fish yearly varied with time and place, but trends were absent. Ages of whitefish in commercial catches, determined by scale reading, revealed three main age groups each year during six summers. Fishing success varied during 7 years and showed no indication of a trend toward better or worse fishing. Some parts of the fishing ground are but lightly exploited. Temperature changes in inshore water masses affected catches in some fishing gear.

INTRODUCTION

Threatened increases in the rate of exploitation of some existing stocks of fish have emphasized the need for sound principles of fisheries management. Toward these ends, the attention of fisheries scientists and other interested people has been drawn to the freshwater lakes as a convenient laboratory in which to formulate fisheries management principles. Accordingly, a study of the Lake Winnipeg fishery began in 1948. The whitefish is common to Lake Winnipeg and to many Canadian lakes, and has a high commercial value. For these reasons, the whitefish fishery of Lake Winnipeg was marked for special attention. The lake supports a considerable whitefish fishery in its northern region. The fishery, which commenced about 1883, has produced an average of about three million pounds of whitefish annually for over 50 years.

The present study deals only with the Lake Winnipeg summer or open-water fishery. A description of it has been published along with some data concerning the lake basin (Kennedy, 1954). The fishery remains essentially as described except that a minority of the fishing boats have been recently (since 1951) equipped with power net-lifters. The 5¼-inch mesh gill-nets are no longer used exclusively. Gill-nets with mesh sizes as small as 4¼ inches, stretched measure, though illegal in the whitefish fishery, have gradually become quite common.

Although season catches during the past few years have been fairly close to the average (Fig. 1), the fishery appears depressed economically, and was recently the object of an enquiry by a Commission appointed by the Manitoba Government. Summer production figures for the past 12 seasons are compared with the average for the latest 20-year period in Fig. 1.

ACKNOWLEDGMENTS

I am indebted to a host of fishermen for their co-operation in sampling their catches, and for other information. Age data for 1948 and 1949 were supplied by

[1]Received for publication March 5, 1958.
[2]Present address: Blueberry Mountain, Alberta.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

Dr W. A. Kennedy. The age determinations were made by Miss L. Dawn Brown and Mr W. A. Musker. Mr K. G. Roberts assisted in the collection and arrangement of most of these data. To these, and to the many summer workers, I offer thanks.

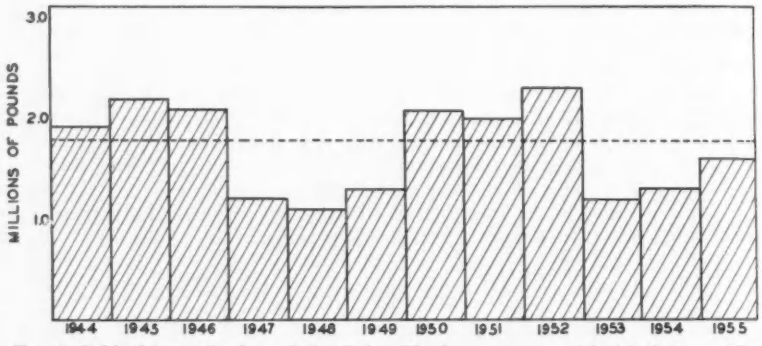

FIG. 1. Whitefish production of the Lake Winnipeg summer whitefish fishery. The broken line indicates the average for the period 1936–1955. Calculations were based on Manitoba Government records.

STUDY METHODS

For convenience, the lake was divided into seven study areas. Their boundaries are shown on the accompanying map (Fig. 2). Each area is designated by a letter. Only Areas A, B, C, and D are contained within the legal boundaries of the summer whitefish fishery.

The fishing port of Mukutawa River was selected as a suitable place at which to study the whitefish fishery, since more than 20 of the 130 to 150 boats engaged were usually based there, and could be easily contacted. The adjacent fishing grounds are representative of Area B, along whose eastern shore are based 65% of the fishing boats.

Investigators were based at Mukutawa River each summer during the whitefish season. Their duties included daily interviews with the captain of each fishing boat to record the details of the day's fishing. The investigators recorded the location of the nets, the mesh size, the number of yards of nets fished, and the time interval between lifts. They recorded the captain's report on the type of bottom, the depth of the water, and the amount of fish caught, including those not offered for sale. These data were recorded daily on cards provided for the purpose, for each boat based at the port. By 1952 it was evident that boats based at Mukutawa River seldom fished in any but Area B; data from other areas were few. Accordingly, in 1953 one investigator was based for a time in each of Areas C and D to record catch statistics from Areas A, C, and D. In spite of the increased effort, data from Areas A and C are inadequate in numbers.

In addition to catch statistics, representative samples of the fish caught were taken during half-month periods. The fish were selected at random from the

catches while the boats were being unloaded, and weighed individually on a small spring scale. It was practical to sample only those species regularly landed in considerable quantity.

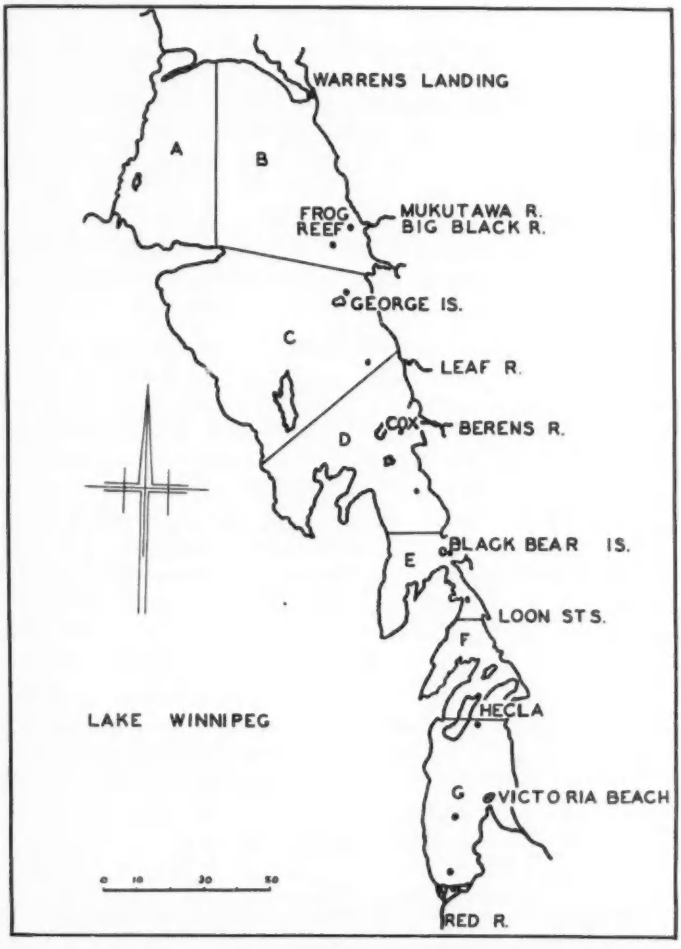

FIG. 2. Lake Winnipeg, showing statistical subdivisions which are designated by letters and temperature stations.

Bathythermograph readings at 11 sampling stations along the main north-south navigation route were recorded at intervals during each summer beginning in 1950. The stations were located from 10 to 30 miles apart over a distance of about 200 miles. The five northern stations were located on the whitefish grounds as shown in Fig. 2.

In addition to these temperature data, a series of water temperature observations were recorded from inshore waters at each of two fishing ports, namely Berens River and Mukutawa River (Fig. 2). These observations were made using a maximum-minimum thermometer, and only surface and bottom temperatures were recorded.

During 1948 and 1949, gill-nets identical with those used in the fishery were fished by the investigators on the fishing grounds near the port of Mukutawa River. Specimens of whitefish thus captured were sampled for age determination. The commercial catches from the same area during 1951 to 1953, inclusive, furnished the specimens for age determinations during those years. Age determinations were made by the scale reading method.

During these years, accumulated data were presented in detail in a series of manuscript reports, which are on file at all Fisheries Research Board stations. The present study attempts to combine these data in order to assess any trend or change which may have occurred in the stocks of fish.

THE CATCH

The following species of fish appeared regularly in the catches during each year:

Lake whitefish	*Coregonus clupeaformis*
Burbot	*Lota lota*
Sucker	*Catostomus* spp.
Cisco	*Leucichthys* spp.
Yellow walleye	*Stizostedion vitreum vitreum*
Sauger	*Stizostedion canadense*
Northern pike	*Esox lucius*
Yellow perch	*Perca flavescens*
Freshwater drum	*Aplodinotus grunniens*

Total recorded catch (Table I) refers to observed catches by investigators who recorded only the production at the ports at which fishermen were interviewed. It was estimated that the interviews involved more than 90% of the fishing effort at Mukutawa River, and hence about the same proportion of the production there. In all, production and other figures in this study are descriptive of about 5% of the fishery during most years and as high as 20% during 1953.

TABLE I. Percentage composition of the recorded catch of a representative segment of the summer whitefish fishery on Lake Winnipeg. A plus sign indicates less than 1%.

	White-fish	Burbot	Cisco	Sucker	Yellow walleye	Sauger	Pike	Perch	Drum	Recorded catch
	%	%	%	%	%	%	%	%		10^3 *lb*
1948	41	28	17	12	+	+	+	+	0	300
1950	34	49	7	8	+	+	+	+	0	700
1951	54	17	14	7	5	1	+	+	0	400
1952	51	18	12	9	5	2	1	+	1	600
1953	38	24	13	17	4	1	3	+	0	885
1954	31	24	18	18	6	2	+	+	0	634
1955	30	47	13	3	5	1	1	+	0	165

Rank in abundance of the various species present in the catches was similar over the seven years. Whitefish usually ranked first and made up about one half of the catch. Burbot ranked second except in 1950 and again in 1955 when catches were nearly one-half burbot. This is not a commercial species and is, therefore, a considerable nuisance. Burbot appeared on some fishing grounds each year in quantities sufficient to discourage fishing there (Hewson, 1955). During each year ciscoes usually ranked third by weight in abundance in the catches, and suckers fourth. Most of the suckers were *Catostomus commersoni* although numbers of the species *C. catostomus* appeared. Yellow walleye appeared in considerable quantities during the last five years probably because the fishermen tended to fish more inshore. This species amounted to 5% to 6% by weight of the recorded catches from 1951 to 1955. Sauger, northern pike, and yellow perch combined varied from 2% to 5% by weight during the seven seasons.

SIZE COMPOSITION OF THE CATCH

Since the major part of the fishing effort was expended in Area B, most of the sampling was done there as well. Whitefish samples there totalled 783 fish in 1948 and during following years totals ranged from 1,200 to more than 3,000 fish from the area.

In order to assess whether apparent changes in average weight during a year are real, "F" values (Snedecor, 1946) were calculated for the series of averages during each of the years 1951, 1952 and 1953 (Table IV; from data of Table II). These three years were selected because data were most numerous. The "F" value for 1951 was 27.17 and proved significant at the 1% level; that for 1952 ($F = 17.51$) was significant at the 5% level, while $F = 0.97$ for 1953 was not significant. These tests indicate that during 1951 and 1952 at least, the different average weights observed reflect real changes.

TABLE II. Some statistics for Area B (Lake Winnipeg) whitefish for June and July, 1951–1953. SX = weight in pounds; n = number in sample.

		June 1–15	June 16–30	July 1–15	July 16–31	Totals
SX	1951	278.5	1964.6	1849.8	1267.1	5360.0
	1952	1540.2	1295.7	1450.6	1512.6	5799.1
	1953	1746.1	1307.8	472.9	1057.1	4583.9
SX^2	1951	631.47	4757.12	4856.68	3307.51	13552.78
	1952	3865.60	3219.55	3616.22	4049.28	14750.65
	1953	4421.53	3319.90	1187.37	2642.95	11571.75
$\frac{(SX)^2}{n}$	1951	610.73	4567.64	4674.54	3087.58	12917.99 12940.49 14195.67
	1952	3765.42	3074.79	3489.61	3877.89	14207.71 10898.41
	1953	4182.25	3126.76	1118.17	2472.25	10899.43
n	1951	127	845	732	520	2224
	1952	630	546	603	590	2369
	1953	729	547	200	452	1928

Similar "F" tests were applied to the July average weights from 1948 through 1955 (Table IV; from data of Table III). These indicated that the observed differences in average weights at comparable times in different years were probably real. $F = 38.65$ (July 1–15) and $F = 28.49$ (July 16–31) were significant at the 1% level.

TABLE III. Some statistics for Area B (Lake Winnipeg) whitefish for July, 1948–1955. SX = weight in pounds, n = number in sample.

Date, July		1948	1950	1951	1952	1953	1954	1955	Totals
SX	1–15	853.1	624.1	1849.8	1450.6	472.9	1299.1	3317.4	9867.0
	16–31	1270.1	1095.6	1267.1	1512.6	1057.1	1080.7	670.1	7953.3
SX^2	1–15	2469.81	1517.29	4856.68	3616.22	1187.37	2986.21	8657.60	25291.18
	16–31	3560.61	2736.68	3307.51	4049.28	2642.95	2617.97	1675.65	20590.65
$\frac{(SX)^2}{n}$	1–15	2332.63	1475.38	4674.53	3489.62	1118.17	2845.97	8092.02	24028.32 23956.12
	16–31	3424.95	2638.11	3087.58	3877.90	2472.26	2511.64	1570.05	19582.49 19529.17
n	1–15	312	264	732	603	200	593	1360	4064
	16–31	471	455	520	590	452	465	286	3239

Evidence for a trend in the average size of whitefish in Area B (Table IV) seems absent. The high figure of 2.7 lb for July 1948 appears atypical of the group as a whole. This average was calculated for fish that were taken 15 to 30 miles offshore, whereas during subsequent years, nearly all the fish were taken

TABLE IV. The average size of whitefish sampled in Area B during seven summer seasons, in pounds. The number of fish in each sample is shown in parentheses. The "F" values test the averages of their rows, or columns, for differences in mean size; they are calculated from the data of Table II and III. When $P = 0.05$–0.01, F values are marked *; when $P < 0.01$, F is marked **.

Year	June 1–15	June 16–30	July 1–15	July 16–31	Aug. 1–15	"F" value
1948	...	...	2.73 (312)	2.70 (471)	...	...
1950	...	...	2.36 (264)	2.41 (455)	2.3 (522)	...
1951	2.19 (127)	2.33 (845)	2.53 (732)	2.44 (520)	...	27.17**
1952	2.44 (630)	2.37 (546)	2.41 (603)	2.56 (590)	...	17.51*
1953	2.39 (729)	2.39 (547)	2.36 (200)	2.34 (452)	...	0.97
1954	...	2.1 (463)	2.19 (593)	2.32 (465)	...	...
1955	... ...	2.3 (1873)	2.44 (1360)	2.34 (286)	...	...
"F" value	...	...	38.68**	28.49**	...	...

from 1 to 15 miles offshore, and the averages proved to be a good deal lower. These findings substantiated the impression of the investigators that the smaller fish were more common in inshore waters.

July averages of 2.4 lb during 1950 fell to 2.3 lb the following month. It is possible that the reduction in size was due to exploitation by the fishery, but during 1951, the average size tended to increase in spite of exploitation by the fishery, having reached 2.5 lb by July 1–15. Had this apparent upturn been due to seasonal growth of the fish, it would be reasonable to expect a similar pattern during 1952 and subsequent years. This did not occur. During 1952 and again in 1953, the

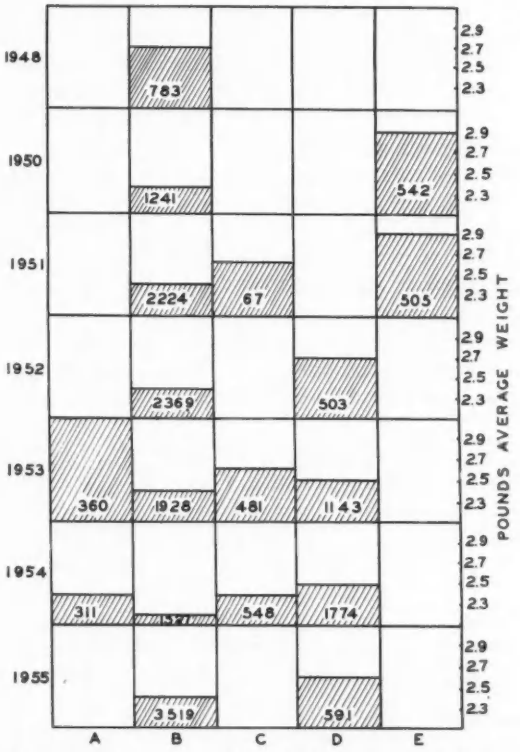

FIG. 3. The average weight of various numbers of summer caught whitefish from 5 areas on Lake Winnipeg.

averages remained fairly static at 2.4 lb except during July 16-31 when a sharp rise to 2.6 lb occurred in 1952, and a fall to 2.3 lb was noted in 1953. The 1954 series suggests that gains may be attributable to seasonal growth, but the series for 1955 does not.

Although all possible care was taken each year to sample only fish caught in the standard 5¼-inch-mesh nets, the marked increase in the illegal use of smaller

meshed nets during 1954 and 1955 may have introduced some bias. This circumstance makes the averages of 2.1 and 2.2 lb in 1954 somewhat suspect. On the other hand, the catches were mainly from fishing grounds well inshore where smaller fish seemed quite common.

In any case, there is no clearly defined pattern in the variability of average size. The erratic variations observed may be explained by assuming that the population is composed of many discrete groups and that "catchability" of each group changes erratically, possibly because the groups move vertically and horizontally almost at random. Kennedy (1954) gives evidence that Lake Winnipeg whitefish tend to occur in discrete groups and that catchability varies.

Similar data for four additional areas were recorded but they are too few to warrant detailed comparison and only season average sizes are presented. Figure 3 shows the average size of whitefish sampled from five areas during seven summer seasons. During 1951 and 1952 whitefish appeared in the catches of the walleye fishery in Area E in numbers sufficient to permit sampling, and these averages are presented for comparison with those of the whitefish fishery.

Figure 3 indicates an average size of 3.0 lb for Area A whitefish during 1953 and a drop to 2.4 lb during the following year. The catches were from widely separated fishing grounds, and one sample was taken inshore. In these circumstances, the averages are probably not comparable. For similar reasons, the apparent drop in average size in Area C during 1954 is not considered.

Area D samples averaged 2.7 lb in 1952, and fell to 2.5 lb during 1953 and 1954, but rose again to 2.6 lb during 1955. These averages are comparable, and the differences indicated are probably real. The reasons for the changes are presumedly similar to those already noted for similar changes in Area B.

TABLE V. The average size in pounds of four species sampled from the whitefish fishery in Area B. (Number of specimens in parentheses.)

	Burbot	Cisco	Longnose sucker	Yellow walleye
1948	...	0.7 (239)	...	...
1952	4.4 (114)	0.6 (575)	3.1 (292)	1.9 (691)
1953	3.7 (101)	...	...	2.0 (76)
1954	3.5 (1428)	...	...	...

Area E samples averaged 2.9 lb during 1951 and 1952. It is of interest that these fish appeared in the catches at a fairly large size in spite of the fact that they were sampled from nets of 3¾-, 4½-, as well as 5¼-inch mesh.

The average size of four species is shown in Table V. The average size of burbot ranged from 4.4 lb in 1952 to 3.5 lb in 1954. Ciscoes averaged 0.7 and 0.6 lb during two years, and longnose sucker sampled during 1952 averaged 3.1 lb. Yellow walleye averaged 1.9 lb in 1952 and 2.0 lb in 1953.

AGE COMPOSITION OF THE CATCH

The commercial whitefish catches were made up almost entirely of fish ranging in age from IV to VI years, inclusive, with age V usually predominant (Table VI). The average weight at each of these three ages varied considerably with the locality at which they were taken. At Mukutawa River (Area B), 4, 5 and 6 year olds averaged approximately 2.0, 2.5 and 2.6 lb respectively whereas in Area E the averages were nearly a half pound heavier at each of these ages. The variation in the weight of individuals at a given age was considerable, and apparent in all localities.

TABLE VI. Numbers of Lake Winnipeg whitefish of each age (completed years) sampled from 5¼-inch-mesh gill-nets.

Year	Sampled at	Age III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	Total
1948	Mukutawa River	5	14	114	63	7	3	...	...	...	...	...	...	206
1949	Mukutawa River	3	98	10	5	2	...	...	...	...	...	...	...	118
1950	Mukutawa River	10	106	351	16	6	3	0	1	...	...	...	...	493
1951	Mukutawa River	4	446	402	153	16	6	1	2	...	...	...	...	1030
	Warrens Landing	0	128	245	122	6	1	...	...	...	...	...	...	502
	Loon Straits	23	202	157	153	4	1	1	0	1	1	...	...	543
1952	Warrens Landing	1	41	402	64	9	...	...	...	...	...	...	...	517
	Mukutawa River	4	44	360	99	18	3	2	...	...	...	...	...	530
	Berens River	2	23	379	78	22	2	...	...	...	...	...	...	506
	Loon Straits	14	33	385	56	19	...	...	...	...	...	...	...	507
1953	Warrens Landing	5	118	98	268	25	4	1	0	0	1	...	...	520
	Mukutawa River	13	246	110	115	24	1	1	1	...	...	...	...	511
	Berens River	0	287	90	165	19	8	0	1	0	1	0	1	572
	Total	84	1786	3103	1357	177	32	6	5	1	3	0	1	6555

Although whitefish in the commercial catches ranged in age from III to XIV years, fish of ages other than IV, V or VI years occurred in negligible numbers. For convenience, fish younger than age IV were included with that group, and the age VI group includes a few fish older than age VI. Thus three groups are formed: age V, those older, and those younger. The age composition of some whitefish samples grouped in this manner is shown in Table VII.

The samples represent fish from three areas. The samples designated B were taken near Mukutawa River, and those termed B1 were taken near Warrens Landing in Area B. Samples termed D and E were taken near Berens River and Loon

TABLE VII. The age composition, in percentage, of some whitefish catches from three areas on Lake Winnipeg, compared with season average weights. B = Mukutawa River, B1 = Warrens Landing, D = Berens River, E = Loon Straits.

	Age V				Younger than V				Older than V				Av. weight (lb)		
Area	B	B1	D	E	B	B1	D	E	B	B1	D	E	B	D	E
1948	55	...	...	...	10	...	...	...	35	...	...	...	2.7	...	...
1949	8	...	...	...	86	...	...	...	6	...	...	...	2.2	...	...
1950	71	...	...	...	24	...	...	...	5	...	...	...	2.4	...	...
1951	39	49	...	29	44	25	...	41	17	26	...	30	2.4	...	2.9
1952	68	78	75	76	9	8	5	9	23	14	20	15	2.4	2.7	2.9
1953	21	19	16	...	51	24	50	...	28	57	34	...	2.4	2.5	...

Straits, respectively. The 1948 sample totalled 206 fish and the 1949 samples totalled 118 fish. All other samples totalled nearly 500 fish in each instance. The average weight for whitefish in 1949 is based solely on the 118 fish just mentioned, while average weight during other years included fish sampled at random from the commercial catches for which age determinations were not made.

Age V fish were generally the dominant age group in the catch from Area B (Table VII) but in two years, 1949 and 1953, age IV was dominant. It is assumed that 1949 was exceptional because of a strong year-class which were age IV that year; the fact that the same year-class appeared stronger than usual as age V in 1950 and age VI in 1951 adds support to this assumption. There is also evidence that in Area E there was a strong year-class that appeared as age IV in 1951 and age V in 1952.

The proportion of fish older than V years that were present in the catches remained quite high over the 6-year period. These figures indicate a steady escapement of age V fish and are indicative of a stable population. Indeed, strong year-classes did not appear to influence fishing success (Table X).

It is of interest that the average size of the fish taken in the fishery was influenced so little by quite different combinations of the three age groups evident during some years. For example, the average size in Area B during 1950 was the same as that noted in 1953 in spite of the fact that 76% of the sample in 1950 was made up of fish age V and older compared with only 49% for these two groups in 1953. Other examples are apparent as well, notably for Area E, where two samples average 2.9 lb during consecutive years. In 1951, 41% of the sample were younger than age V while only 9% were younger than age V in 1952. In the light of these data, therefore, average size alone is but a poor statistic with which to compare stocks of fish such as these from one year to another.

Theoretically, a sample from a lightly exploited population of whitefish should contain a higher proportion of larger (older) fish than that for a heavily exploited population because the fishing gear is probably more efficient for larger fish. Assuming a negligible difference in growth rates between two populations, and other factors being equal, it is therefore possible to compare degrees of exploitation by the proportion of older fish present in the samples. Comparing columns B and B1 in Table VII by age-groups reveals, in general, a greater proportion of age V fish, fewer younger fish, and more older fish in the B1 (Warrens Landing) sample. These data indicate that the B population (Mukutawa River) was the more heavily exploited of the two.

FISHING EFFORT

As already mentioned, about 65% of the fishing boats were based in Area B, and most of the fishing effort was concentrated in the vicinity of the home ports. Since 1949, Mukutawa River boats rarely fished more than 12 miles off shore and there is reason to believe that other boats in the area were similarly deployed. Net-sets in other areas were also characteristically inshore, but recorded data of the location of these are few, by comparison. Inshore fishing was expedient during bygone years when sail boats were used. It is probable that the present choice of the traditional inshore grounds has been influenced by habit.

Heretofore, gill-nets were customarily lifted daily, weather permitting, and net lifts after more than one night in the water were rare. During more recent years the lift interval has tended to increase. Some data regarding the lift interval during seven summer seasons is shown in Table VIII together with a measure of the observed fishing effort. As already mentioned, fishing effort shown is not necessarily proportional each year, hence total fishing effort comparisons between years should be made with reservations. In 1948 one-night lifts made up 70% and two-night lifts 13% (Table VIII). The remaining 17% of the effort was partly one-night, partly two-night, sets and could not be classified for any specific lift interval. Since 1948 the proportion of one-night lifts has dropped sharply. During 1955 only 40% of the nets could be classified as one-night lifts. Although the increased lift interval permits the use of more gear, and theoretically larger catches, any advantage was probably offset by the resulting poorer quality of the product.

TABLE VIII. Recorded fishing effort expressed as thousands of 100-yard gill-nets lifted once, and classified by time in the water.

Year	One-night lifts	Two-night lifts	Unspecified	Total effort observed
	%	%	%	1000's *of lifts*
1948	70	13	17	28.9
1950	80	10	10	37.1
1951	69	22	9	31.2
1952	68	19	13	36.1
1953	63	16	21	49.0
1954	50	5	45	63.6
1955	40	22	38	6.8

INDEX OF FISHING SUCCESS (FS INDEX)

A measure of the fishing success of the various species was derived by dividing the catch in pounds by the number of 100-yard long nets employed in their capture. For expediency, this "FS index" (Kennedy, 1956) was calculated using only data from one-night lifts. On theoretical grounds, the FS index as here defined should provide a good measure of the relative abundance of fish actually present in the lake from year to year, since the fishing gear and the techniques remained fairly constant. However, there are indications that the FS index of Lake Winnipeg whitefish may vary for reasons other than abundance (Kennedy, 1954). For this reason, FS indices here are not necessarily regarded as indices of abundance.

TABLE IX. Fishing success indices for whitefish from the Lake Winnipeg summer fishery during 4 half-month periods.

	June 1–15	June 16–30	July 1–15	July 16–31
1948	3	4	3	6
1950	4	6	8	4
1951	6	5	8	5
1952	10	5	7	5
1953	4	4	4	4
1954	1	3	6	3
1955	...	6	8	4

Fishing success for whitefish (Table IX) appeared to improve during the last half of June, taking all years into account, and was generally highest during the first half of July. Indices during the latter part of July approximated those of the latter half of June.

Within each of the four areas (Table X), fishing success varied a good deal from year to year. Indices for Areas A and D during some fortnightly periods (not shown) were frequently high, but were generally not sustained, so that in summary, they are similar to those for Area B. Catches usually fell off markedly in Area D early in July, and the boats based there moved to existing bases in Area B. Area

TABLE X. Indices of fishing success for whitefish from four areas in Lake Winnipeg. Figures based on less than 100 units of effort are marked with an asterisk.

	A	B	C	D
1948	6	4	2	4
1950	0	6	2	6
1951	13	6	8	0*
1952	6	8	0.5*	7
1953	4	4	3	5
1954	...	4	3	5
1955	...	7	...	...

A was usually not visited by most of the boats under observation until catches began to fall off at fishing grounds closer to their home ports (Area B), and then visits were only sporadic. Area C indices too are based on data recorded mainly for occasional exploratory sorties by fishermen mostly from bases in Area B, who were easily discouraged from making the long runs into unfamiliar waters.

OTHER SPECIES. Table XI shows indices of fishing success for whitefish compared with that for all species combined. From 1948 to 1952, the index for combined species was roughly twice that of the whitefish index. During 1953 to 1955, the combined species index was nearly three times the other. During these last three seasons, fishermen landed quantities of rough fish formerly discarded, particularly burbot, in order to collect a "bounty" offered by the Manitoba Government. These circumstances probably account for the apparent over-all increase in the fishing success index.

TABLE XI. Indices of fishing success for whitefish and for all species of fish combined during seven summer seasons.

	1948	1950	1951	1952	1953	1954	1955
Whitefish	4	6	6	7	4	4	7
Combined species	9	12	11	14	14	14	21

WATER TEMPERATURES AND WHITEFISH CATCHES

Temperatures (on file) recorded regularly at the 11 stations along the main navigation route during three summers showed a marked similarity between surface and bottom. Thermal stratification was not pronounced. When differences existed, they were usually in the order of 1° to 5°F, and occurred mainly in the upper half

of the water column. Depth ranged from about 4 fathoms at the Red River station to 9 fathoms at most of the northern stations. Maximum temperatures at neighbouring stations were similar each year, but at a given time a gradation could be detected between extreme stations. As might be expected, the more southerly stations reached their maximum temperatures before those in the north.

At Mukutawa River, near-maximum temperatures occurred about the middle of July. Bottom temperatures varied during three years from 58°F to 65°F at this time. Maximum fishing success coincided more or less with the attainment of maximum temperatures, but data were too scant to establish a precise relationship. Accordingly, during the summer of 1954 the two series of temperature stations were established at Berens and Mukutawa Rivers.

At Berens River, inshore water temperatures at surface and bottom were about 10°F higher than their counterparts at the station farthest offshore, seven miles away. Bottom and surface temperatures at any station differed by two or three degrees usually, and at times coincided. Generally speaking, there was a progressive warming at all stations as the season progressed, but the trend was broken by occasional intrusions of colder water masses at some stations. At Station 8 (not shown) located on a fishing ground, the bottom temperature was 45°F on June 12, 62.5°F about a month later, and 64°F on July 27. Fishing success was highest when the bottom temperature approached 54°F. As water temperature rose above 54°, catches fell off sharply indicating that whitefish had probably left the area.

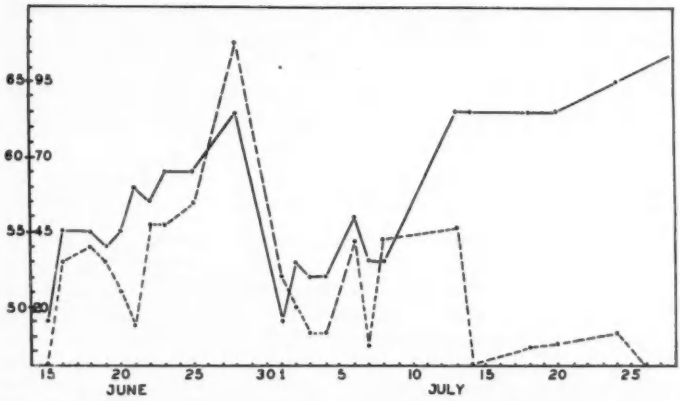

FIG. 4. The relationship between bottom water temperature 1¼ miles shoreward and catches of whitefish in a trap-net 6 miles offshore near Mukutawa River, 1954. *Temperature* (°F), left scale and solid line. *Catches* (pounds per lift), right scale and broken line.

Water temperature observations from seven similarly situated stations off Mukutawa River closely paralleled those of Berens River (Fig. 4). Again, fluctuations in fishing success for whitefish in the commercial fishery adjacent to the seven stations did not appear to be related to the observed temperature fluctuations. As at Berens River, fishing success declined rapidly at a similar water temperature.

About six miles offshore, at the outermost temperature station of the Mukutawa River series, the investigators operated a Lake Erie type trap-net. Whitefish catches in the trap-net appeared to be associated with temperatures at three shoreward stations, 1 to 1½ miles distant. As the temperature fluctuated during June at these stations, whitefish catches fluctuated more or less in harmony until about mid-July after which whitefish scarcely appeared in the trap-net catches. The water temperature 1½ miles shoreward of the trap-net at this time was 63°F.

It appears that the temperature of the water inshore, or adjacent to, a particular fishing ground influences the movement, and therefore catches, of whitefish where trap-net gear is used. The apparent failure of gill-net catches to react in a similar way was perhaps due to the necessity of "averaging" long lengths of gill-nets in order to compare performance with temperature observations. Too, temperature effects may have been modified or obscured by other factors associated with the performance of gill-nets.

SUMMARY AND CONCLUSIONS

During a detailed study of part of the fishery for whitefish on Lake Winnipeg, the fishermen have tended to concentrate more and more in a particular area which has been designated as Area B, to fish inshore to a greater extent, and to leave a larger proportion of their nets for more than one night. The average size of the fish landed from a given area varied erratically and showed no distinguishable trend. The average size of fish varied from area to area; the largest fish tended to be on the less heavily exploited fishing grounds; whitefish caught inshore tended to be larger than those caught offshore. There is evidence that some year classes are stronger than others but variation in year class strength is not a major factor in annual catch. Catches are influenced by water temperatures; a temperature of about 54°F appears to stimulate whitefish to move to colder waters; they seem to avoid altogether temperatures higher than 60°F.

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Growth of Fishes in Different Salinities[1]

By P. Canagaratnam[2]

Institute of Fisheries,
University of British Columbia,
Vancouver, B.C.

ABSTRACT

Sizes attained by several species of fish inhabiting both sea and fresh waters suggest that the larger size of the marine form is due to the higher osmotic content of the medium. This theory was tested experimentally by comparing fresh and saltwater growth of juvenile coho, sockeye and chum salmon and adult goldfish. Such factors as temperature and food were rigidly controlled. In general, the salmonids (coho, chum or sockeye underyearlings) grew more rapidly in saline than in fresh water. Adult goldfish did not show any significant difference in weight increase.

INTRODUCTION

Eleven species of fish occurring in two or three habitats of differing salinity are listed in Table I, together with their sizes at maturity or as otherwise indicated. With one exception, the smelt (*Osmerus mordax*), the marine and brackish water inhabitants are the larger. Some show appreciable differences and warrant the conclusion that the larger members of the species, for some reason, are found in the marine environment. Even among marine species, such as the Atlantic herring (*Clupea harengus*) or the giant perch (*Lates calcarifer*), the larger sizes occur in higher salinities. The Atlantic herring measures from 240 to 350 mm, while the same species occurring in the Baltic measures 160 to 200 mm. The salinity of the

Table I. Sizes of adult fishes living in marine, brackish and freshwater environments. Precise stage of maturity is usually not given in the literature; sizes are mostly those of mature fish taken in a commercial or sport fishery.

SPECIES	MARINE	BRACKISH	FRESH WATER	AUTHORITY
Chanos chanos (milkfish)	...	...	600–1500 mm (Hawaian fresh-water ponds)	Jordan and Evermann, 1903
	1800 mm (Gulf of Mannar, Indian Ocean)	...	...	Munro, 1955
	Over 1500 mm (Indian Ocean)	...	...	Weber and Beaufort, 1913
	1500 mm (Australia estuarine waters)	...	...	Roughley, 1953
	257 mm[a] (Krusadai, India)	403 mm[a] (Mandapam Camp, India)	604 mm[a] (Rameswaram, India)	Chidambaram and Unny, 1946
Clupea harengus (Atlantic herring)	240–350 mm (Atlantic)	160–200 mm (Baltic)	...	Hodgson, 1934

[a]These measurements were taken after only one year's growth of the milkfish in the various experimental ponds.

[1]Received for publication January 7, 1958.

[2]Author's permanent address: Research Officer, Fisheries Research Station, P.O. Box 531, Colombo 3, Ceylon.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

TABLE I: *continued*

SPECIES	MARINE	BRACKISH	FRESH WATER	AUTHORITY
Salmo salar (Atlantic salmon)	480 mm (Nova Scotia)	...	350 mm (Grand Lake, Nova Scotia)	Wilder, 1947
Pomolobus pseudoharengus (alewife)	258 mm	...	145 mm (Lake Ontario)	Pritchard, 1929
Osmerus mordax (smelt)	150–250 mm (New Brunswick)	...	...	McKenzie, 1946
	...	...	150–250 mm	Dymond, 1944
Salvelinus fontinalis (eastern brook trout)	334 mm (New Brunswick)	...	274 mm (New Brunswick)	Wilder, 1952
Coregonus clupeaformis (whitefish)	...	403–466 mm 1359–1812 g	...	Rawson, 1946
	...	479 mm, 2038 g (Redberry Lake, Saskatchewan)	...	Rawson, 1946
	...	...	1132–1585 g (Lake Winnipeg, Manitoba)	Hinks, 1943
Lates calcarifer (giant perch)	263 kg (Bay of Bengal)	27–45 kg (Australian estuaries)	...	Roughley, 1953
	...	1500 mm (Australian estuaries)	...	Munro, 1955
Salmo gairdneri gairdneri (steelhead trout)	1134 mm[b] (British Columbia)	...	...	Clemens and Wilby, 1949
Salmo gairdneri kamloops (Kamloops trout)	...	...	907 mm[b] (British Columbia)	Carl and Clemens, 1953
Oncorhynchus nerka nerka (sockeye)	830 mm (British Columbia)	...	...	Carl and Clemens, 1953
O. n. kennerlyi (kokanee)	...	...	185–245 mm (Kootenay Lake, British Columbia)	Vernon, 1957

[b]The maximum sizes on record.

Baltic varies from 20‰ in the south to 1‰ in some of the northern regions. Hodgson (1934) states that the salinity in regions frequented by Baltic herring is roughly one-seventh that of the Atlantic (which latter is about 35‰). Similarly *Lates calcarifer* of the Indo-Pacific varies in size in different regions (Roughley, 1953), but no records of the salinities, temperatures or productivities of the waters are given. Salinities of "pure" sea water, as well as those of brackish or estuarine waters, vary considerably in the different parts of the world.

Anadromous forms that mature in the sea and their related forms that mature in lakes, present some vivid contrasts. Several species, for which data are available, have been included in Table I. The sockeye salmon of the Pacific coast (*Oncorhynchus nerka nerka*) and its non-migratory relative (*O. nerka kennerlyi*) form

an interesting comparison. Ricker (1940) states that the only known morphological difference between these two forms is the smaller average size, at maturity, of the non-migratory salmon. Non-migratory sockeye consist of two types—the kokanee, progeny of the lacustrine form and the "residual", progeny of the migratory forms (Ricker, 1938). Both forms are distinctly smaller than the migratory sockeye (Table I).

Foerster (1947) showed experimentally that if offspring of non-migratory sockeye or kokanee had the opportunity of going to sea as smolts, then they would grow as large as natural sea-run sockeye. In this experiment he took kokanee eggs from the Kootenay Lake area, British Columbia, then hatched and reared them to yearling size in Smith Falls Hatchery, Cultus Lake, British Columbia. As yearlings, these kokanee (63,874) were marked by clipping off both pelvic fins and were then released in the outlet stream below the lake at a time when the natural sea-run sockeye smolts of Cultus Lake were going to sea. In their fifth year 25 individuals, with the pelvic fins off, were taken in the commercial fishery. The lengths of those recovered were larger than the normal four-year-old Cultus sockeye. In Kootenay Lake kokanee mature at age IV or age III (Vernon, 1957). The size difference between the fifth year "sea-run kokanee" and the Kootenay Lake spawning kokanee (Vernon, 1957) is great enough to suggest that the marine environment promotes better growth.

Some data on size of chinook or "quinnat" salmon (*Oncorhynchus tshawytscha*) in New Zealand are summarized in Table II. These data were kindly supplied by Dr E. Percival (1956). The difference between the average lengths of the sea-run chinook salmon and of the "landlocked" form ranges from 170 to 400 mm. The size of the sea-run form is comparable to the corresponding species on the west coast of Canada. Carl and Clemens (1953) give an average length of 907 mm.

TABLE II. Length and weight of chinook salmon (*Oncorhynchus tshawytscha*) from New Zealand (data obtained from Dr E. Percival).

"Type", place and date		Average length *mm*	Average weight *g*
Freshwater habitat			
"Landlocked" quinnat salmon, Macdonald Creek, Westland			
May 1955		569	
May 1956		546	
Sea-run fish			
Quinnat salmon—Waimakariri	1944	746	4800
" " —Opihi	1944	900	6000

Johnsen (1944) mentions several fish that vary in size in different North European waters. Stickleback (*Spinachia spinachia*), two-spotted goby (*Gobius flavescens*), lanternfish (*Myctophum glaciale*) and plaice (*Pleuronectes platessa*) are some of the species that show great variation in size at maturity. He states that when a species varies in size within its habitat, it usually finds the optimum conditions in places where it reaches the largest size. Temperature and food are mentioned as the chief controlling factors but there is no statement regarding

salinities in the different areas. For instance, *Myctophum glaciale* in the Mediterranean is half the size of that found in the Norwegian waters. The difference in the salinity in the two areas ranges from 5‰ to 10‰ (Sverdrup *et al.*, 1942). *Pleuronectes platessa* in its fifth year ranges from 210 to 400 mm in Iceland and from 170 to 280 mm in the West Baltic. In the West Baltic, the size range for this species is generally smaller for all groups beyond the third year. The salinity in the West Baltic never exceeds 16‰ while in Iceland it is about 35‰. Johnsen stresses only the variations in the temperature and the nutrients in these waters. Hodgson (1934) on the other hand states definitely that the size variation in the Atlantic herring in the Baltic may be due to both temperature and salinity.

Alm (1934) states that landlocked Atlantic salmon never attain as large a size as those that go to sea although they have equally good feeding conditions. Salmon in the Baltic precincts show a preference for the southern waters. Alm (1934) assumes that the warmer waters and higher salinities of the southern Baltic are beneficial and hence the smolts from the northern rivers concentrate in this region. The salinity here ranges from 8 to 10‰ at the surface and from 15 to 20‰ in the deeper parts, whereas the northern part of the Baltic never exceeds 3.5‰. The temperature too increases southwards. The records of the sizes of salmon in the various parts of the Baltic show considerable differences (Dixon, 1934).

Such observations have been made repeatedly. Sverdrup *et al.* (1942) state that among the euryhaline animals, those living in reduced salinities have a smaller maximum size than those of the same species inhabiting higher salinities. Reduced size may result from the scarcity of food organisms, which must be adapted to live in or near the same biotope. These authors state "whatever the cause may be, it should be noted here that it is a strange and unexplained fact that with few exceptions marine animals from groups with freshwater representatives are larger than the freshwater relatives and usually the size difference is enormous".

Gunter (1957) has assembled size data for 14 species of fish which enter brackish or fresh water from the sea and notes the size differences similar to those under discussion here. Gunter considers that the younger (hence smaller) members of the species tend to enter less saline waters and that is probably the cause of the differences observed.

Several experiments suggest that growth is more rapid for certain species of fish when they live in more saline environments. For example, the introduction of freshwater whitefish (*Coregonus clupeaformis*) in 1940 into a saline lake of Saskatchewan (which contained no fish), resulted in a small commercial fishery in 1945 (Rawson, 1946). The salinity of the lake was 15‰. The potential fish food supply was of the same order as that in larger freshwater lakes where whitefish are produced in large quantities. Whitefish were introduced as fry, and after four years measured from 403 to 466 mm and weighed 1359 to 1812 g (Table I). This shows a rate of growth twice that of the same species in such fresh waters as Lake Winnipeg or the Great Lakes (Rawson, 1946). Hinks (1943) states that in Manitoba commercial catches, the average weight of whitefish is within the range 1130 to 1585 g and the bulk of the fish so caught are 6 to 8 years old.

Fish culture work with salmon also indicates better growth in more saline waters. Fraser (1918) reports an experiment in rearing sockeye salmon in fresh water, and compared them with sea-run fish of the Fraser River. The freshwater forms reached an average fork length of 250 mm while the sea-run averaged 566 mm. The growth in the first year in both cases was very nearly the same, but in the second, third, and fourth years there was almost a three-fold difference in the sea-run form.

Some experiments by Gibson and Hirst (1955) are also pertinent to the present discussion. They find that an isotonic solution (physiologically saline solution) produces better growth than fresh water in the pre-adult life of guppies. These workers raised guppies in fresh water for an average period of 10 weeks or until mature, at various temperatures and found that the fastest growth occurred at 23° and 25°C. Above and below these limits the growth was slow. Guppies in 25% sea water and at 30° surpassed the growth rate of the freshwater fish at 25° during a period of 60 to 80 days of age. The fastest growth occurred at 23° and 25% sea water. The fish also flourished in 50% sea water and at 20° or 25°. The growth curve for the one at 20° and 50% sea water was steeper than that for 25% sea water and the same temperature.

These several experiments as well as the comparisons of sizes of fish from environments of different salinity suggest that the higher salt content of the environment may in itself provide better growing conditions. It is, of course, appreciated that in nature food and temperature exert spectacular effects on growth. In the experiments described in this paper an attempt is made to control these factors and to test the hypothesis that higher salinities will favour increased growth in those species which can tolerate a wide range of salinity.

MATERIALS AND METHODS

The juvenile salmon were cultured from eggs in the laboratory. The goldfish were procured from the Goldfish Supply Co. (Stouffville, Ontario). Two series of coho fry (*Oncorhynchus kisutch*) were studied. In the first series, a group (initially 50 fish) was maintained at each of three salinities (0, 6 and 12‰); in the second series, groups of the same size were followed at 0 and 18‰. The initial average size of the first group was 3.6 cm and 0.469 g; that of the second group 4.2 cm and 1.007 g. Groups of sockeye (*O. nerka*) fry (69 in each) were cultured at 0 and 6‰. Their initial average size was 2.78 cm and 0.207 g. The chum salmon (*O. keta*) had been cultured in sea water (about 20‰) prior to the experiments. Groups of 12 fish were maintained at 6 and 30‰. Their initial average size was 6.7 cm and 2.42 g. Goldfish were studied in groups of 15 at salinities of 0 and 6‰. The initial average size was 8.4 cm and 12.07 g.

The fish were kept in glass aquaria. The goldfish were maintained at room temperature (about 20°C). The tanks containing the salmonids were surrounded by a large metal trough of cold running water which maintained temperatures of about 10°C. Groups compared had always experienced the same variations in temperature.

Water of the desired salinity was prepared by diluting sea water or by adding sea salt to sea water. Tanks were cleaned daily and the water completely changed twice per week. All fish except the coho at 18‰ salinity were transferred directly to the desired salinity. These coho were first placed at an intermediate salinity for two days.

The fish were fed a daily ration of 10% of their body weight per day (Barrett and Hurn, 1954). The diet was prepared by mixing canned salmon (60%), ground beef liver (25%), a commercial trout food (12%), pablum mixed cereal (2%), brewers' yeast (1%) and a few drops of cod liver oil.

Although the commercial trout food probably contains the ingredients necessary for good growth, these fish had been previously accustomed to a salmon-liver-pablum mixture and did not at first readily take the commercial dried food. Hence the above mixture was developed and used throughout the experiment. In addition, the salmonids were given a daily supplement of brine shrimp nauplii. Coates and Schwab (1956) report that fish are healthier when given their natural food and that young salmon grew well on brine shrimp nauplii.

The total weight of each group of fish was recorded at the beginning and weekly thereafter. The food for each group (10% of body weight) was fed in three approximately equal amounts, three times a day (8:00 a.m., 11:00 a.m., and 2:00 p.m.). The filtered brine shrimp nauplii were fed to salmon daily at about 5:00 or 6:00 p.m. in an amount proportional (by volume) to the weight of the groups of fish.

To avoid unnecessary strain and exhaustion of the fish during the weighing, a small canvas bag was fitted in the middle of the dip net used to remove the fish from the tanks. The fish were thus collected in the bag which was full of water and transferred into a large beaker which was thus lined with the bag-net. The beaker with fish, water and the bag-net was weighed on a balance which had a sensitivity of 0.1 g. The bag-net was then lifted up to the edge of the beaker and held there for about five seconds so that nearly all the water, except that adhering to the fish drained into the beaker. The fish were then quickly transferred from the bag-net into their tank. The beaker, water and the bag-net were then weighed. The difference gave the weight of the fish. This method of weighing was checked with a group of 50 coho taken from the same stock as the one used in the experiment. An error of only 0.5% was found in the mean when the highest or lowest weights were taken in 25 attempts.

RESULTS

Coho—Series 1

Survival of these coho was good and the numbers remained constant after the second week. The initial average weights of the 50 fish in each tank were very nearly equal. The percentage increase (Fig. 1) was highest in the 12‰ salinity tank at both stages (end of the fifth and the tenth weeks). The percentage increase on the initial average weights in the 0, 6 and 12‰ salinities at the end of the fifth week was, 77.2, 88.1, 155.8; at the end of 10 weeks it was 215.1, 251.2, 421.5 respectively.

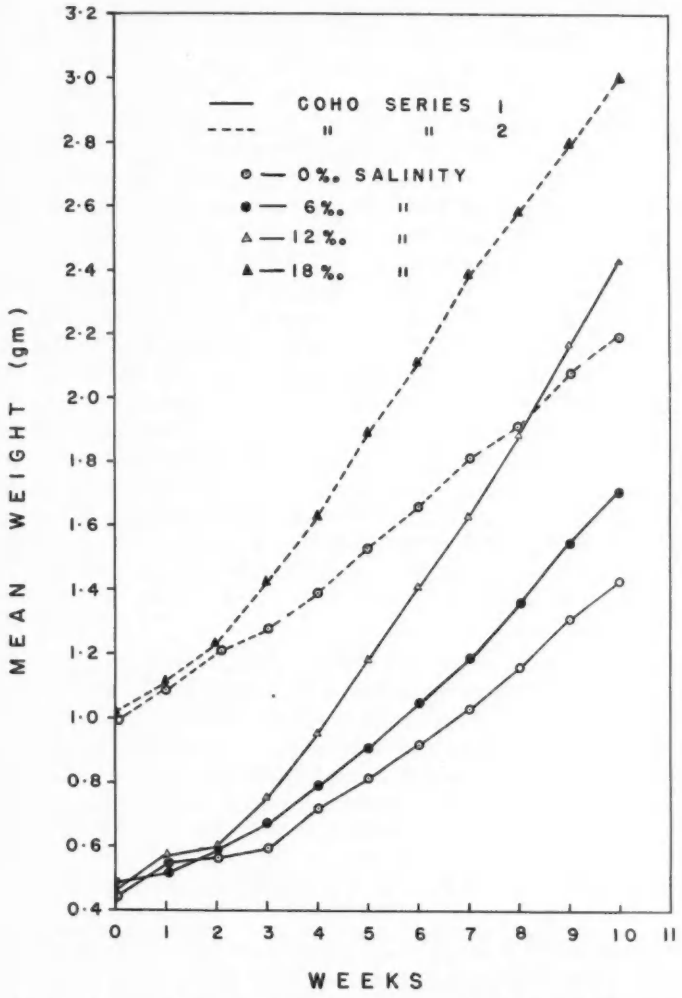

FIG. 1. Mean weekly weight of coho, Series 1 and 2, in fresh water and in the various salinities.

COHO—SERIES 2

The second series of coho was used to observe the effect of a higher salinity (18‰). Again the fish survived well. The average initial weight in each tank (0 and 18‰ salinity) was a little over twice that of the average initial weight of Series 1. The percentage increase was again greater in the saline environment. In Fig. 1 the growth curves for the freshwater groups of both series are almost

parallel. The curves for the higher salinity have greater slopes, indicating faster growth.

Sockeye and Chum Salmon Fry

These fish did not survive well in the small aquaria and the data are too few for significant conclusions. The results are, however, similar to those obtained for coho. With the sockeye in fresh water the mean weight at the beginning was 0.192 g for 69 fish. At the end of 5 weeks the average reached 0.560 g for the 10 surviving fish. This gave a 192% increase over the initial average. At the end of the tenth week there were only 8 left and the average weight was 0.806 g, an increase of 320%. There was a somewhat better survival (35%) of sockeye fry in 6‰ salinity. After 5 weeks there was only a 117% increase in weight for the 24 fry remaining in the 6‰ salinity tank, which was thus considerably lower than the 192% increase of sockeye in fresh water for the same period. At the end of the 10 weeks the percentage increase (336%) was slightly higher than the 320% increase of the freshwater ones. The number of sockeye in this tank, after the first week, remained constant throughout the experimental period.

The chum salmon experienced very high mortalities but this is not attributed to the salinity since the experimental conditions for this group of fish were far from ideal (small size of the tanks). The percentage increase at the end of 5 weeks was 166% for 11 fish in 30‰ salinity as compared with 120% for 8 fish in 6‰ salinity.

Goldfish

Goldfish were used to test the effect of salinity on a purely freshwater type. Comparison of growth was made only in fresh water and 6‰ salinity, since higher salinities (15‰ or greater) are lethal to goldfish (Black, 1951). The 15 fish in each of the two tanks were mature and any appreciable increase in growth was not anticipated. At the end of the fifth week the percentage increase in fresh water was slightly higher (1.7%) but at the end of the tenth week the ones in 6‰ salinity showed a very small increment over that of the freshwater group (0.7%). These differences are not considered significant.

DISCUSSION

The literature survey as well as the experimental findings support the hypothesis that several species of fish will grow better in more saline waters although they may thrive well in waters of different salt content. All the salmon data obtained in the present experiments are in general agreement, although significance can be attached only to the coho results. Although the theory is supported by facts from many sources, the mechanism responsible for greater growth in saline environments remains to be demonstrated. Variable osmotic stress and effects of osmoregulation are the most obvious lines for explanation. However, growth is a many-sided phenomenon and the real explanation may eventually be found elsewhere.

Growth of fish is governed by two factors—heredity and environment. Inherited characters are influenced to a marked extent by favourable or unfavourable environmental features, particularly in the early stages of growth. These effects may

be superficial (*e.g.* colour) or deep seated (*e.g.* changes in meristic counts and size). Of the influencing environmental factors, temperature, salinity and the food supply are probably the most important. In the present experiments every effort was made to control temperature and food.

It has been suggested that the freshwater fish must expend more energy than marine fish to maintain osmotic equilibrium. Experimental evidence to date is circumstantial but the passage of large amounts of water through tissues might create extra demands either on kidney or salt-absorbing mechanisms. In particular it has been suggested that the demands for thyroid hormone may be greater when large quantities of osmotic water must be removed and, in consequence, the marine fish—normally adjusted to an environment containing much iodine—may find its iodine-trapping thyroid mechanism excessively stimulated and be unable to turn out the amounts of thyroid hormone required for growth as well as for osmoregulation (Hoar, 1957). Some species, such as the freshwater goldfish or the anadromous smelt, may have such an efficient thyroid apparatus that adequate quantities of hormone can be produced even in low iodine waters, while others such as the alewife (*Pomolobus pseudoharengus*) may survive only when all other conditions are relatively favourable (Hoar, 1952).

Studies of the physiology and endocrinology of fish growth will be required to test these theories and to show how salinity exerts its effect on growth. The present data only provide evidence that some species of fish which can withstand a wide range of salinity will grow better in the more saline environment.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr W. S. Hoar, for suggesting the problem and supervising the work. He also wishes to thank Dr W. A. Clemens and Dr C. C. Lindsey for their valuable suggestions and criticisms, Mr D. F. Alderdice of the Biological Station, Nanaimo, British Columbia, for supplying the chum salmon used in this experiment, and Dr P. A. Larkin, Dr E. J. Crossman and Mr D. E. McAllister for their useful criticisms.

This work was made possible through an award of a Colombo-Plan Scholarship by the Technical Co-operation Service, Department of Trade and Commerce, Ottawa, on an application made by the Government of Ceylon.

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A Study of Six Winter Seasons of Commercial Fishing on Lake Winnipeg, 1950–1955[1]

By L. C. Hewson[2]

Fisheries Research Board of Canada
Biological Station, London, Ontario

ABSTRACT

Investigations of a Lake Winnipeg winter fishery for sauger, *Stizostedion canadense*, and yellow walleye, *S. vitreum vitreum*, during January and February each year revealed some significant changes in average size for two species as the season progressed, and from year to year. Average weight based on 50 to 1600 fish approximated 0.5 lb for sauger and 0.7 for walleye in most areas. Fishing success declined during the study period. A sample of the sauger catch revealed mainly age-groups III, IV and V. Fish catches appeared unrelated to either water temperatures or dissolved oxygen. Increased use of nylon nets had no apparent effect on annual production.

INTRODUCTION

This study was motivated by the need for basic biological data upon which fisheries management principles are based and was concurrent with a similar study of the summer fishery.

Specifically, the object of this study was to record the average size of the species taken in the commercial fishery, to describe the fishing gear and fishing methods, and to record a measure of the fishing success. The simplicity of the study was desirable in view of the extensive area of the fishery, the limited shelter available to the investigators, and the hazards of the winter climate.

The Lake Winnipeg Winter Fishery

From the standpoint of regulations, the fishery by 1950 had evolved five separate component parts. This process of evolution continued during the years of the study, as evidenced by official changes in boundaries and mesh sizes, confounding the investigation to some degree, but had little effect on the fishery itself. In these circumstances, it is expedient to ignore official boundaries and mesh sizes altogether, and to describe the fishery from the point of view of the investigator.

The fishery as studied occupied most of the lake south of a boundary line roughly westward from Pigeon Point (Fig. 1) and encompassed the area in which most fishermen were engaged. The mesh size used in this great area was predominantly 3 inches (stretched measures) but the extremes encountered whether legal or otherwise were $2\frac{1}{2}$ and $5\frac{1}{4}$ inches.

Most of the winter fishermen on the southern part of the lake live in or near the villages and towns of Winnipeg Beach, Gimli, Victoria Beach, Camp Morton and Hecla. In the central part, several fishermen live in the hamlets of Pine Dock,

[1]Received for publication March 11, 1957.
[2]Author's present address: Blueberry Mountain, Alberta.

J. Fish. Res. Bd. Canada, 16(1), 1959.
Printed in Canada.

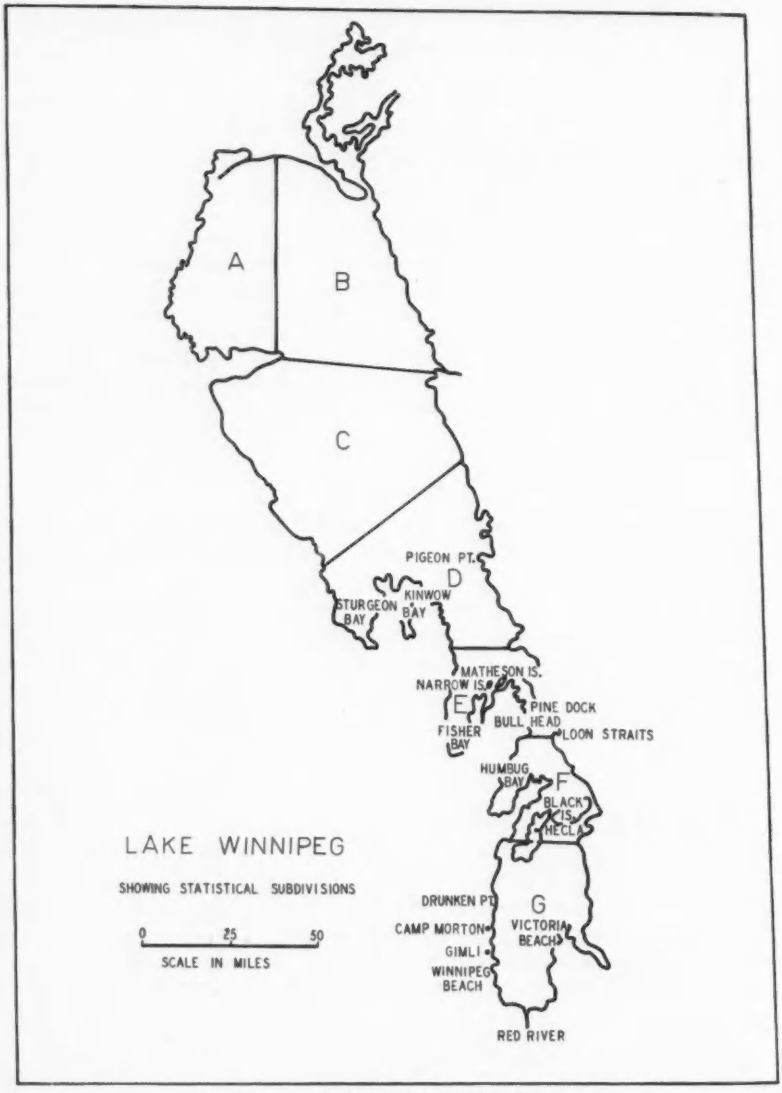

FIG. 1. A map of Lake Winnipeg.

Loon Straits and Matheson Island. The fishing grounds adjacent to these settlements were most consistently exploited, as opposed to the fishing grounds adjacent to the many camps which serve as bases for the fall and winter fisheries.

The winter fishing camps are quite numerous north of Hecla Island. Typically, they are very roughly built and are inadequate for family living and are occupied

almost solely by the fisherman and his helpers. The dwellings are usually of log construction and are heated with wood-burning sheet-metal stoves. The rough hand-built furniture is the utmost in austerity. A small shelter housing a vehicle is common to most, and sometimes, a storage shed. Some of these camps are accessible by a winter road, especially those along the west shore from Humbug Bay to Matheson Island, and some others in Fisher Bay and Sturgeon Bay. There are no roads at all along the east shore. Fishermen there who wish to begin the winter operations as soon as the ice forms, and others who can not depend on travel by land, reach their camps by water in the late fall with a food supply, and "freeze-in".

The presence of a persistent ice cover is common to the entire winter fishery. The lake ice along the shores and in sheltered bays is usually strong enough to support a man's weight as early as November 16. The ice cover thickens rapidly to a winter maximum of 2 to 4 feet, depending upon snow cover, and persists until April or May.

It is important to the fishery that a safe ice cover form quickly, for concentrations of fish are common at this time. During some years, the early ice has been suddenly broken up by high winds occasioning the loss of considerable amounts of gear. Further, heavy snows on weak ice cause slush to form and render travel extremely difficult.

The Winter Fishing Vehicle

These conveyances are substitutes for the skiffs and vessels used during open water. They carry the fisherman's gear, serve as shelters, and haul his catch.

One of the earliest conveyances was the hand-drawn sled, and it is still in evidence at "first ice" when heavier vehicles are unsafe. The first refinement was the introduction of the carriole drawn by a dog train; these too, are still used by a few fishermen. Later, the sled was enlarged and a small shelter built upon it, to be pulled by a single horse. Still later, the shelters were enlarged to cabooses as large as 8 by 14 feet. These were mounted on bob-sleighs and furnished with stoves and other household equipment and were drawn by a team of horses. In due time, a variety of tractors replaced the horses. Finally, Bombardier snowmobiles appeared, both as tractors and as units complete in themselves.

These latter vehicles vastly increased the efficiency and mobility of the winter fisherman but their high initial cost has limited the number in use. Their owners usually defray some of the cost by limited custom freighting.

Some Economic Factors

Most of the capital costs in the winter fishery are borne by the fisherman. Typical tractor-equipped outfits employ a crew of three: the operator and two hired hands, each of whom receives a fixed wage of approximately $125 a month and board rather than a share of the catch. A three-man crew fishing for sauger would employ about 150 nets, each about 100 yards long.

Although the initial cost is high, wear and tear on winter nets is relatively light, and they should last 10 years or more. The total capitalization for this type of outfit has been estimated variously from $3000 to $5000.

There is frequently enormous variation in the fisherman's income, from week to week and during different years, so accurate estimates of seasonal income are difficult to make. Daily income for the outfit just described has been observed to vary from $3 to more than $100. When gross income falls lower than $20 to $30 daily, all but a few fishermen cease operations. The average crew seems capable of lifting only about 15 nets per day; thus a return of $1.35 to $2.00 per net is imperative.

In the more remote parts of the fishing grounds it is uneconomical for the fisherman to market his catch daily because of the prohibitive distances to the shipping points. These fishermen of necessity freeze their fish, box them, and deliver their winter's catch in large consignments, usually only one or two in a season. The fish are hauled to market by tractor trains.

The tractor train or "swing" is made up of several heavy-duty bob-sleighs coupled together. In their function, they resemble railway flat cars. The train is complete with a caboose at the rear for the crew, and is drawn by a Diesel track-type tractor equipped with a snow plow. On its return journey the train brings supplies for the fishermen and goods for the trading posts in the northern parts of the lake.

Each year since 1950 more fishermen have sold their catch "fresh" (non-frozen). The price is usually higher for non-frozen fish, and it eliminates some of the risk of loss due to a price slump when, on occasion, fishermen have lost the opportunity of marketing much of an entire season's catch of frozen fish. Non-frozen fish are more in demand partly because the buyer can be more certain of quality, and partly because of consumer demand.

Fishermen who market fish in the "fresh" condition store their catch in the heated caboose while lifting their nets, and allow catches to accumulate in sheds ashore. Consignments are transferred to shipping points every 2 or 3 days by Bombardier snowmobile or by truck along a winter road.

Fishing Methods

The setting of gill nets under the ice cover is accomplished initially with a "jigger" (Fig. 2), an ingenious hand-operated device whose construction and use have been described by Sprules (1949). Briefly, it is a submarine device made from a plank about 8 feet long. A suitably arranged system of levers translates a backward pull on its control line into forward motion. It accomplishes the feat of placing a line under the ice for a considerable distance (usually 100 yards) by using only two openings. This "running line" serves to pull a gill net into position between the two openings.

In practice, a series of nets are set end to end so that any two nets in the series have a common anchor stone. The anchor stone is marked by a line reaching vertically to the surface of the ice and attached there to a small stake set into the ice. The lifting process has been fully described by Kennedy (1956); however, the rudiments of the operation are as follows:

A hole is punched at each end of a net a little to one side of the anchor stakes to avoid cutting the vertical line. At one hole, a hooked iron rod serves to locate

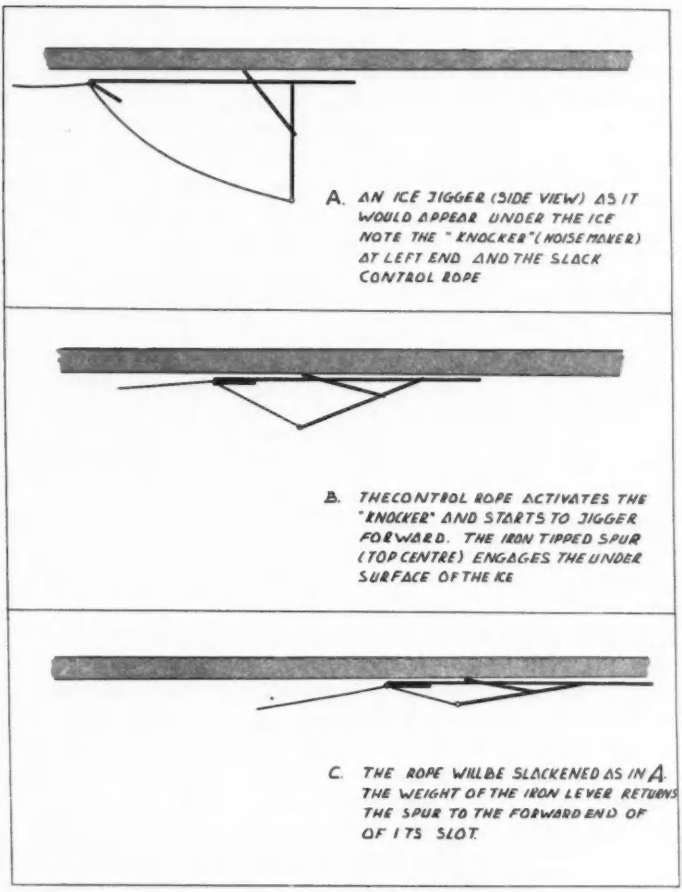

FIG. 2. A diagram of an ice jigger in operation.

this line with which to lift the anchor stone sufficiently to expose the attachment of the bridle of the gill net. A turn or two of the vertical line around the marker stake holds the anchor stone suspended. The "running line", a few yards longer than the net, is attached to the gill net bridle, and the net is then allowed to re-settle for the time being. At the other hole, a second anchor stone is similarly retrieved, the bridle secured, and the net is pulled out a few yards at a time. This allows the fish to be removed before the mesh freezes. The running line now occupies the former position of the net. A man retraces the distance to the former hole and by means of the line is able to replace the gill net. The running line is untied, the bridles re-attached, the anchor stones dropped, and the net remains as before.

During the winter season on Lake Winnipeg, the position of the nets in relation to the bottom is frequently varied. Flat stones are substituted for some

of the conventional lead weights on the bottom of the net, and attached with a length of strong twine 6 to 10 feet long. The net may be set on the bottom as well by winding up the attaching strings, using the stone weight as a spool. On other occasions, gill nets are set immediately under the surface of the ice. Alternate pairs of corks and leads are removed, and inflated toy rubber balloons on two foot ties are attached at 6 fathom intervals on the cork line. The balloons if frozen to the underside of the ice, readily break as the net is removed, and prevent loss or damage to the net.

METHODS OF COLLECTING DATA

Areas

For study purposes the fishing grounds were divided into 7 convenient areas (Fig. 1) and the time into half-month periods. Two investigators, working together, and travelling on the ice with a Bombardier snowmobile attempted to visit each of Areas D, E, F, and G at least once during each half-month period. As many fishermen as possible were visited in each area throughout their working day. These four areas extend throughout a distance of about 120 miles, and it was sometimes difficult to visit each area within the specified period due to delays caused by poor travelling conditions or bad weather. For these reasons, fishing operations in Areas A and C were excluded from the study. Area B is fished only during the summer.

Within each statistical area one or more bases were chosen from which to make daily sorties in search of fishermen upon the ice. In Area G the bases used were Gimli and Winnipeg Beach since many fishermen operated from this vicinity. Data representative of Area G were recorded mainly from Drunken Point southward to the Red River. Occasional sorties to the eastward of mid-lake were made but fishing effort there was relatively light and the trips unrewarding.

From the village of Hecla in Area F it was a short run to one of the main fishing grounds along the south shore of Black Island. Fishermen occupied the grounds up to 5 miles offshore on the east side of Hecla Island as well. During 1950 to 1952 there was a considerable fishery along the west shore of Humbug Bay. These operations were accessible from one of the winter camps along the shore.

Bases in Area E were located on Narrow Island in Fisher Bay, near Bull Head and at Loon Straits. During most years fishing operations practically ceased toward the latter part of January in this area.

Suitable bases in Area D were difficult to establish. For the most part, the camps were widely dispersed and occupied only for a short time during some years. Most were too small to accommodate overnight visitors except one at Kinwow Bay and another at Sturgeon Bay. As a consequence data for Area D may not be entirely representative.

The Daily Interview

Fishermen usually began lifting their nets in the early forenoon, and the investigators began their visits to each crew at this time. As the nets were cleared, each fish was weighed on a spring scale and its weight recorded together with the length of the net, in yards and its width in meshes, the mesh size, the kind of

twine and the elapsed time since it had been last lifted. Fishermen were asked to report the number of nets lifted the previous day in terms of the foregoing details, and to state or estimate the catch in pounds for each species. Most fishermen were able to supply these data with considerable accuracy and gave it with little or no reservation. The investigators attempted to visit all crews within reach of the "home" base, and to weigh a random sample of each species from each crew. This usually required two or more days to accomplish. On stormy days and on days of limited visibility it was difficult to locate fishermen on the ice.

Limnology

Some limnological data were recorded during the study from 3 stations. These were located 2 miles north of the Red River Lighthouse, at a depth of 4 fathoms, 2 miles east of Gimli, and 2 miles west of Loon Straits at depths of 5 fathoms. Quantitative analyses of dissolved oxygen and free carbon dioxide were made for bottom and surface water samples from these three stations. Temperatures for each sample were determined with a reversing thermometer or a rod thermometer. Oxygen determinations were made using Miller's method (DeLaporte, 1920) and those for carbon dioxide were made by titrating NaOH into a measured sample using phenolphthalein indicator.

OBSERVATIONS

Composition of the Catch

The catches each year were predominantly sauger and yellow walleye (Table I). These two species combined made up 50 to 70% of the total and were the mainstay of the fishery. The catch of cisco, *Leucichthys* spp., ranged from 4 to 31% but contributed little to the fisherman's income due to a limited demand for them. Northern pike, *Esox lucius,* and yellow perch, *Perca flavescens,* were taken only in small quantities and together did not exceed 5% during any season. Burbot, *Lota lota,* which were sold as mink feed or discarded altogether, made up from 3 to 17% of the catch; suckers, mainly *Catostomus* spp., comprised from 1 to 4%. Freshwater drum, *Aplodinotus grunniens,* were marketable only during 1953 through 1955 when they made up 12 to 23% of the catch. The increase in their catch, therefore, does not reflect any increase in abundance. A similar situation exists in the statistics shown for cisco. They are taken in quantity only in Area D from which little effort was recorded during the years in question, hence the decreased poundage taken does not indicate reduced abundance.

Table I. Percentage composition of some Lake Winnipeg winter fishery catches by weight; and the total catch examined, in thousands of pounds.

	Sauger	Yellow walleye	Cisco	Burbot	Sucker	Pike	Perch	Others	Fresh-water drum	Catch examined
	%	%	%	%	%	%	%	%	%	10^3 *lb*
1951	43	28	22	3	...	...	1	3	...	39
1952	29	22	31	8	4	2	3	1	...	34
1953	36	24	5	17	+	+	2	+	16	17
1954	40	17	4	14	1	+	+	1	23	15
1955	60	4	7	13	1	+	3	+	12	5

Average Size

The average weight of sauger in random samples from the commercial catches recorded at half-month intervals during each of 6 winters is shown in 4 areas in Table II. For the most part, the average weight varied between 0.5 and 0.7 lb. In general the average weight was highest in Area D, followed by that for Area G. The average weights of sauger in the other two areas, E and F, were smaller. However, the validity of comparisons of average weight between areas is questionable since the mesh size of the gill nets from which the samples were taken varied between areas (Table III) and may have biased average size. Nevertheless, average size of sauger in Area D, at least, is believed to be greater than that in the three other areas. The larger average size could result from the fact that Area D is but lightly exploited when compared with the areas south of it. Movements of sauger into Area D from regions to the north probably occur, and these fish would be

Table II. The average weight, in pounds, of sauger sampled from Lake Winnipeg winter fishery catches. The number in each sample is shown in parenthesis. Letters refer to the regions of the lake shown in Fig. 1.

	January 1–15				January 16–31				February 1–15				February 16–28			
	D	E	F	G	D	E	F	G	D	E	F	G	D	E	F	G
1950	...	...	...	0.662 (68)	...	0.524 (95)	...	0.634 (64)	...	...	0.605 (119)	0.560 (135)	...	0.621 (56)	0.591 (67)	0.615 (122)
1951	...	0.597 (468)	0.606 (520)	0.584 (406)	0.700 (409)	0.497 (470)	0.511 (481)	0.561 (457)	...	0.479 (358)	0.511 (473)	0.506 (466)	...	0.622 (421)	0.556 (253)	0.536 (326)
1952	0.741 (98)	0.501 (51)	0.529 (457)	0.666 (168)	0.750 (63)	0.526 (56)	0.497 (168)	0.638 (326)	0.709 (260)	...	...	0.641 (134)	0.675 (80)	...	...	0.555 (179)
1953	...	...	...	0.582 (231)	0.784 (25)	0.547 (259)	0.547 (267)	...	0.657 (66)	...	...	0.606 (169)	...	...	...	0.616 (30)
1954	...	...	...	...	...	...	0.490 (151)	0.548 (288)	...	...	0.486 (248)	0.461 (213)	...	...	0.492 (356)	0.469 (412)
1955	...	...	...	...	...	...	...	...	0.641 (188)	...	0.530 (253)	0.508 (440)	...	...	...	0.555 (43)

Table III. Recorded mesh size (in inches, stretched measure) of numbers of 100-yard-long gill nets sampled from the Lake Winnipeg winter fishery.

	Mesh size	Area D		Area E		Area F		Area G		Total	Percentage mesh size each year
	inches	*no.*	*%*	*no.*	*%*	*no.*	*%*	*no.*	*%*	*no.*	*%*
1951	under 3	0	0	113	32	24	6	1	<1	138	8.8
	3	13	29	151	43	406	94	741	99	1311	83.3
	over 3	32	71	87	25	1	<1	4	<1	124	7.9
1952	under 3	0	0	9	7	98	36	0	0	107	7.9
	3	90	28	45	37	175	64	622	100	932	69.3
	over 3	236	72	68	56	0	0	0	0	304	22.6
1953	under 3	0	0	0	0	75	50	0	0	75	11.3
	3	26	100	109	100	75	50	375	100	585	88.6
	over 3	...	...	...	...	...	...	...	...	...	...
1954	under 3	...	...	82	100	65	20	0	0	147	16.2
	3	...	...	0	0	249	74	490	100	739	81.5
	over 3	...	...	0	0	21	6	0	0	21	2.3
1955	under 3	6	75	...	...	82	54	0	0	88	30.3
	3	2	25	...	...	70	46	130	100	202	69.6
	over 3	...	...	...	...	...	...	...	...	...	...
All years	under 3	6	2	204	31	344	26	1	<1	555	11.6
	3	131	32	305	46	975	73	2358	99	3769	79.0
	over 3	268	66	155	23	22	1	4	<1	449	9.4

essentially a virgin population since sauger fishing gear (3-inch mesh) is not used there.

Average weight of sauger sampled from each area varied with time, and a fairly consistent pattern of variation was suggested in most years, especially in Area

TABLE IV. Some statistics for weights (in pounds) of Lake Winnipeg sauger samples from the winter fishery in Area G. SX = sum of individual weights; SX^2 = sum of squares of weights; $\bar{x}$ = mean weight; n = number of specimens.

	January 1–15				January 16–31				February 1–15				February 16–28			
	SX	SX^2	$\bar{x}$	n	SX	SX^2	$\bar{x}$	n	SX	SX^2	$\bar{x}$	n	SX	SX^2	$\bar{x}$	n
1950	45.0	32.66	0.662	68	40.6	27.62	0.634	64	75.6	45.52	0.560	135	75.1	50.23	0.615	122
1951	237.4	150.36	0.584	406	256.4	156.58	0.561	457	236.2	131.48	0.506	466	174.9	107.23	0.536	326
1952	112.0	79.10	0.666	168	208.4	142.90	0.638	326	85.9	59.83	0.641	134	99.5	62.43	0.555	179
1953	134.5	84.15	0.582	231	...	...	...	...	102.4	65.58	0.606	169	18.5	11.93	0.616	30
1954	...	...	...	...	157.9	93.05	0.548	288	98.3	49.15	0.461	213	193.3	96.89	0.469	412
1955	...	...	...	...	...	...	...	...	223.7	124.29	0.508	440	23.9	13.97	0.555	43

G where records were most complete (Table IV). The differences among all 20 entries (half-months) of this table are of course highly "significant" (F = 30.0); and the average standard deviation *within* half-months, 0.1634, can be used to test individual pairs of mean values, approximately. The variability between all half-months includes differences between years and differences within each fishing season. In general, average size fell somewhat from January to mid-February, then rallied in the latter half of February—though 1952 is an exception.

To test these seasonal differences, the 1950–52 data were adjusted to a constant sample size of 100 by increasing or decreasing SX and SX^2 proportionally, thus permitting orthogonal comparisons, as shown in Table V. (The harmonic mean sample size in these years was 154, but since the number of residual degrees of freedom is more than ample, the round number was used for convenience.) The differences between times of year prove significant in spite of the fact that the procedure used tests only for *parallel* seasonal differences among the three years; 1952's somewhat aberrant behaviour did not mask the main pattern described above, but it is responsible for an interaction mean square (between times of year and gears) that is not too far from the 5% level (Table V).

TABLE V. Analysis of variance of weights of sauger in Area G for 1950–52, the data being adjusted to a sample size of 100 fish each half month.

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	Confidence levels 5%	1%
Total	42.02550	1199				
Between all half-months	3.08950	11	0.280864	F = 8.57**	2.40	3.60
Within half-months	38.93600	1188	0.032774			
Between years	1.49575	2	0.747875	F = 22.8*	19.5	99.5
Between half-months within a year	1.02277	3	0.340923	F = 10.4*	8.53	26.1
Interaction	0.57098	6	0.095163	F = 2.90	3.67	6.88

Similar data for walleye shown in Table VI indicate the tendency for average size to drop as the season progressed. Again, data for areas other than Area G are somewhat inadequate but the larger average size of 1 to 2 lb in Area D is evident. On these fishing grounds, where fishing pressure is not so intense and

TABLE VI. The average weight in pounds of yellow walleye sampled from Lake Winnipeg winter fishery catches. The number in each sample is shown in parenthesis.

	January 1–15				January 16–31				February 1–15				February 16–28			
	D	E	F	G	D	E	F	G	D	E	F	G	D	E	F	G
1950	...	...	...	0.984 (98)	...	1.023 (55)	...	0.921 (125)	...	...	0.772 (59)	0.863 (100)	...	0.953 (45)	0.822 (53)	0.827 (72)
1951	1.177 (244)	0.875 (211)	0.839 (192)	0.762 (256)	0.901 (92)	0.962 (208)	0.574 (95)	0.741 (341)	1.25 (196)	0.892 (269)	0.775 (128)	0.662 (614)	...	0.862 (48)	0.737 (312)	0.675 (409)
1952	1.698 (71)	0.425 (4)	0.685 (142)	0.767 (164)	1.887 (122)	1.258 (62)	0.822 (71)	0.723 (307)	1.501 (189)	...	...	0.738 (139)	1.506 (164)	...	...	0.831 (32)
1953	...	...	...	0.722 (184)	1.050 (4)	0.824 (33)	0.856 (39)	...	1.098 (50)	...	...	0.762 (150)	...	...	...	0.737 (69)
1954	...	...	...	...	...	...	0.823 (52)	0.886 (128)	1.223 (17)	...	0.737 (81)	0.803 (82)	...	...	0.795 (72)	0.725 (119)
1955	...	...	...	...	...	...	...	...	0.961 (31)	...	0.739 (46)	0.733 (51)	...	...	...	...

where movements from unexploited areas are likely to occur, the average size is larger, and larger mesh nets are therefore practical.

In Area G where average size ranged between 0.6 and 0.7 lb (Table VII), decreases in the half-monthly averages occurred during most seasons. During the last three seasons, 1953 to 1955, good comparisons were limited by the small number in the samples. In these years fishermen were scarce and catches of walleye light. Under these conditions the changes in average size may have been more pronounced than the data suggest.

TABLE VII. Some statistics for weights of Lake Winnipeg walleye samples from the winter fishery in Area G. (See Table IV for explanation of symbols).

	January 1–15				January 16–31				February 1–15				February 16–28			
	SX	SX^2	$\bar{x}$	n	SX	SX^2	$\bar{x}$	n	SX	SX^2	$\bar{x}$	n	SX	SX^2	$\bar{x}$	n
1950	96.5	99.01	0.984	298	115.2	118.20	0.921	125	86.3	76.61	0.86	100	59.6	50.76	0.827	72
1951	195.1	160.23	0.762	56	252.9	245.11	0.741	341	406.4	289.52	0.662	614	276.4	203.80	0.675	409
1952	125.8	106.92	0.767	164	222.1	170.47	0.723	307	102.7	81.93	0.738	139	26.6	27.86	0.831	32
1953	132.9	110.55	0.722	184	...	...	...	...	114.3	118.03	0.762	150	50.9	45.85	0.737	69
1954	...	...	...	...	113.5	117.51	0.886	128	65.9	68.17	0.803	82	86.3	72.43	0.725	119
1955	...	...	...	...	...	...	...	...	37.4	48.18	0.733	51	...	...	...	...

These observed declines in average size for both sauger and walleye are probably due to sustained fishing pressure, in view of their evident recovery at the beginning of each new year.

During the early years of the study, 1951 to 1953, some other species appeared in the catches in quantities sufficient to permit sampling. Average weights, together with the year and number in sample were as follows: cisco (1951) 0.5 lb, 865 fish; in 1952, 0.5 lb, 576 fish; yellow perch (1953) 0.4 lb, 190 fish; northern pike (1952) 1.4 lb, 47 fish; burbot (1952) 1.4 lb, 235 fish. Although burbot are quite plentiful in the lake (Hewson, 1955) the small mesh nets (3-inch) used at this season are inefficient for burbot and few are taken.

AGE COMPOSITION

Winter catches were not sampled for age composition. Instead, age data are shown for a total of 652 sauger sampled at random from commercial catches at 4 localities in Areas E, F and G during the autumn of 1950. Most, if not all, these fish were taken in 3-inch mesh nets and are considered comparable to fish taken during the winter season. Sample size, average length and weight for each age group (completed years) are shown in Table VIII.

TABLE VIII. The average weight in pounds (W) and length in inches (L) of Lake Winnipeg sauger, at various ages during the autumn of 1950. Number of specimens is shown in the "N" columns; age is shown in years completed: hence an age II fish had experienced three growing seasons.

	II			III			IV			V			VI			VII			VIII			Total
	N	W	L	N	W	L	N	W	L	N	W	L	N	W	L	N	W	L	N	W	L	
		lb	*in*		*lb*	*in*		*lb*	*in*		*lb*	*in*		*lb*	*in*		*lb*	*in*		*lb*	*in*	
Black Bear Island	1	0.40	9.5	12	0.42	10.35	18	0.48	10.81	16	0.75	12.21	8	0.95	13.02	3	1.16	13.86	...	...	...	58
Red River Mouth	21	0.45	10.63	89	0.55	11.37	119	0.61	11.73	50	0.70	12.12	17	0.70	12.09	1	1.30	14.50	1	2.0	16.0	298
Gull Harbour	...	...	...	15	0.52	11.40	99	0.68	12.05	78	0.76	12.49	29	0.85	12.87	7	1.01	13.32	...	...	...	228
West Doghead	...	...	...	...	...	...	14	0.60	11.75	27	0.77	12.50	23	0.87	12.97	4	1.05	13.47	...	...	...	68
Combined Populations	22	0.45	10.58	116	0.53	11.27	250	0.63	11.79	171	0.74	12.36	77	0.83	12.74	15	1.07	13.55	1	2.0	16.0	652

The samples from each of the 4 localities indicated in Table VIII were similar in age composition and probably represent a single population. Nearly 38% of the combined samples were age IV and 26% were age V. The next larger group, age III were about 18% followed by age VI at about 12%. The remaining 6% were distributed in age II, VII and VIII.

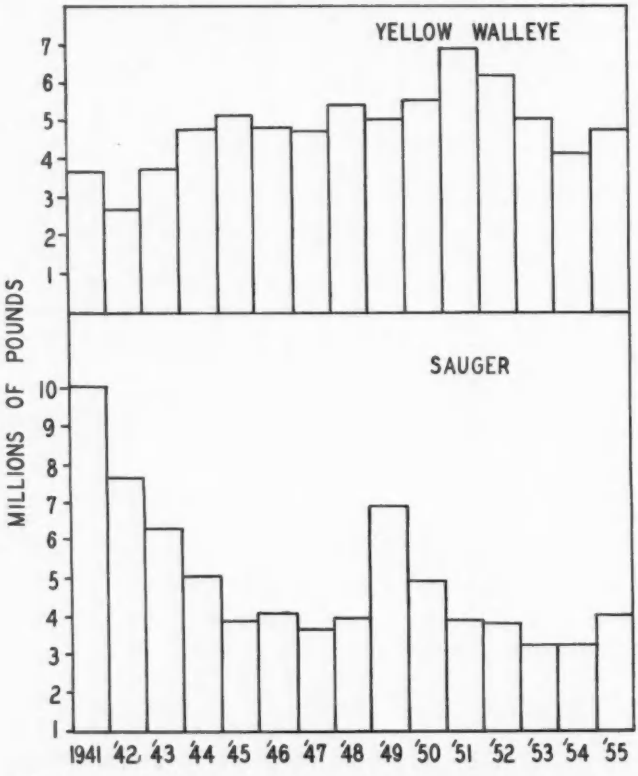

FIG. 3. Recorded annual production of two species from Lake Winnipeg during 15 years. Statistics courtesy of the Manitoba Government.

According to Manitoba government records, sauger production on Lake Winnipeg (Fig. 3) was at a peak during 1949 and was still unusually high during 1950. In 1950 the dominant group was age IV fish followed fairly closely by age V. One may only surmise that the unusual production in 1949 was also dominated by these two year-classes.

INDEX OF FISHING SUCCESS

A measure of fishing success, the FS index (Kennedy, 1956), was calculated each year. The index is the number of pounds produced per 100 yards of net per

lift. No adjustment was made for the time interval between lifts, which remained fairly constant at about 9 days throughout the study period.

FS indices for sauger and walleye for the 4 areas during 5 winter seasons are listed in Table IX. The highest FS values were generally recorded for sauger, and

TABLE IX. Lake Winnipeg winter-season fishing-success (FS) indices (pounds per 100 yards of net per lift).

	Area D		Area E		Area F		Area G	
	Sauger	Walleye	Sauger	Walleye	Sauger	Walleye	Sauger	Walleye
1951	17	15	15	7	10	5	9	8
1952	10	7	5	5	8	6	6	5
1953	24	8	9	4	12	4	10	10
1954	...	...	6	<1	8	3	6	3
1955	19	6	...	...	13	0.6	6	0.5

ranged from 24 in Area D to a low of 5 in Area E. Walleye values ranged from 15 in Area D to less than 1 in Area E. FS indices were notably higher in Area D than in other areas, and generally lowest for Area E. Indices fell off sharply during 1954 and 1955 from the highs recorded in 1953.

The FS index probably does not accurately reflect relative abundance between areas or abundance within an area. In the first place, a concentration of fishing effort in Area F and G tended to reduce the index although fish were apparently plentiful. Secondly, the index values suggest that fish in Area E were quite scarce; however, in this area and to a less extent in Area F, much of the gear was fished at a grave disadvantage. During each January there appeared a water bloom of the diatom, *Melosira varians*. Strong currents, prevailing over much of the fishing grounds in these two areas, carried the bloom through the nets and occluded them sufficiently to render them ineffective. Nets in Areas G and D were not so seriously affected.

In Table X all areas are combined and comparisons of FS indices for sauger, walleye, and combined species may be made. There is a strong downward trend in fishing success for walleye, from 7 in 1951 to 0.6 in 1955. The maximum for sauger was 11 in 1951. The index fell no lower than 7 during any year and rested at 10 in 1955. For all fish, the index value 17 in 1954 and 1955 represented an appreciable decrease from the previous figures of 25 (1951) and 26 (1952, 1953). These indices fell in spite of the increase in the proportion of nylon nets used in the

TABLE X. Lake Winnipeg winter season FS indices, for all areas combined, and the relative utilization of nylon and cotton gill nets.

				Nets in use	
	Sauger	Walleye	All fish	nylon	cotton
				%	%
1951	11	7	25	16	84
1952	7	6	26	34	66
1953	9	6	26	51	49
1954	7	3	17	60	40
1955	10	0.6	17	64	36

fishery. It has been shown (Hewson, 1952) that nylon nets in the Lake Winnipeg fishery are considerably more efficient than cotton nets. This fact notwithstanding, annual production (Fig. 3) since 1951 has shown a downward trend as well. Whether production would have shown a sharper downward trend in the absence of nylon fishing gear is a matter for speculation.

TABLE XI. Some winter limnological data from Lake Winnipeg. Depths shown for the three stations are in fathoms.

		January 1952		February 1952		February 1953		February 1954	
		Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface
2 miles	O_2, cc/litre	8.7	9.1	9.5	8.7	8.3	6.2	8.5	9.2
E. of Gimli,	CO_2, ppm	7	13.5	14	12	10	6	13	10
depth 5 fath.	Temp., °F	33	33	35	33	34.2	32	32.4	32
		March 6, 1953		March 25, 1953		March 12, 1954		March 31, 1954	
2 miles N. of	O_2, cc/litre	11	...	15	15	10	10.6	9.2	10.4
Red River mouth,	CO_2, ppm	10	...	17	17	20	13	25	22
depth 4 fath.	Temp., °F	32	31.6	33.1	32.4	34	34	34.7	34.7
		January 1952		February 1952		February 1953		...	
2 miles W. of	O_2, cc/litre	11.5	10.3	11.6	10.2	9.0	10	...	...
Loon Straits,	CO_2, ppm	13.5	7.5	13	13	13	14	...	...
depth 5 fath.	Temp., °F	34	34	32	33	33.4	32	...	...

SOME LIMNOLOGICAL DATA

Amounts of dissolved oxygen and free carbon dioxide (Table XI) appeared unrelated to fish catches. Dissolved oxygen was in good supply, varying between 7 and 12 cc/litre, and free carbon dioxide varied between 6 and 14 ppm.

Remarkably similar temperatures were noted for bottom and surface samples. Differences less than 2 degrees Fahrenheit were frequently noted, and at times, bottom and surface temperatures coincided. Temperatures for all samples ranged between 31.6 and 35.0°F.

CONCLUSIONS AND DISCUSSION

1. Fishing pressure was sufficiently intense to produce some significant changes in the average size of sauger and walleye when calculated for half-month periods. The downward seasonal trends probably represent local depletion, more or less temporary, in view of the significant rally in average size observed for sauger during two seasons.

2. Significant year-to-year differences in average size of the walleye and sauger populations were found. These differences probably reflect varying complements of the different year-classes making up the commercial catches. Lacking sufficient data of this nature, and a measure of annual fishing pressure, their meaning must remain obscure.

3. For a given species, it is probably unsafe to gauge abundance between years or areas on the basis of fishing success. However, this index probably does reflect the abundance of one species in relation to another of similar size and character during any year. On this assumption, sauger increased relatively at the expense of the walleye.

4. The increasing use of more efficient nets for walleye and sauger is not reflected in annual production figures.

5. Catches each year are not visibly influenced by temperature, oxygen or carbon dioxide in the water; these remain fairly constant from year to year.

ACKNOWLEDGMENTS

I am indebted to Mr K. G. Roberts who helped me collect and arrange most of these data. The cooperation of the many fishermen whose catches we examined is greatly appreciated. I am particularly grateful to those fishermen who extended us their hospitality. My reliance upon Dr W. E. Ricker for certain statistical procedures is gratefully acknowledged.

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